Procise®

Protein Sequencing System

Advanced Operation User's Manual

Applied Biosystems

For Research Use Only. Not for use in diagnostic procedures.

Applera Corporation is committed to providing the world's leading technology and information for life scientists. Applera Corporation consists of the Applied Biosystems and Celera Genomics businesses.

Contents

6 Chemical Optimization

7 PTH Separation

A Function Listing

B Cycle, Method, and Gradient Listings

C Procedure Listings

D Getting Help

E Warranty

Index

1 Introduction and Safety

Overview

About This Chapter This chapter describes the user attention words used throughout this manual and safety issues pertaining to Applied Biosystems instrumentation.

In This Chapter This chapter contains the following topics:

Safety

Documentation User Attention Words

Five user attention words appear in the text of all Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below.

Note Calls attention to useful information.

IMPORTANT Indicates information that is necessary for proper instrument operation.

⚠ CAUTION Cautions the user that a potentially hazardous situation could occur, causing injury to the user or damage to the instrument if this information is ignored.

⚠ WARNING Warns the user that serious physical injury or death to the user or other persons could result if these precautions are not taken.

⚠ DANGER Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Chemical Hazard Warning

⚠ WARNING **CHEMICAL HAZARD**. Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

- Read and understand the material safety data sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with and inhalation of chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Do not leave chemical containers open. Use only with adequate ventilation.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Hazard Warning

⚠ WARNING CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

- Read and understand the material safety data sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Handle chemical wastes in a fume hood.
- Minimize contact with and inhalation of chemical waste. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or protective clothing).
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Site Preparation and Safety Guide

A site preparation and safety guide is a separate document sent to all customers who have purchased a Applied Biosystems instrument. Refer to the guide written for your instrument for information on site preparation, instrument safety, chemical safety, and waste profiles.

About MSDSs

Some of the chemicals used with this instrument may be listed as hazardous by their manufacturer. When hazards exist, warnings are prominently displayed on the labels of all chemicals.

Chemical manufacturers supply a current MSDS before or with shipments of hazardous chemicals to new customers and with the first shipment of a hazardous chemical after an MSDS update. MSDSs provide you with the safety information you need to store, handle, transport and dispose of the chemicals safely.

We strongly recommend that you replace the appropriate MSDS in your files each time you receive a new MSDS packaged with a hazardous chemical.

⚠ WARNING CHEMICAL HAZARD. Be sure to familiarize yourself with the MSDSs before using reagents or solvents.

Ordering MSDSs

You can order free additional copies of MSDSs for chemicals manufactured or distributed by Applied Biosystems using the contact information below.

<table>
<tr><th>To order MSDSs...</th><th>Then...</th></tr>
<tr><td>Over the Internet</td><td>Go to our web site at:
www.appliedbiosystems.com/techsupport
a. Click on MSDSs
b. Enter keywords (or partial words), or a part number, or the MSDSs Documents on Demand index number.
c. Click on Search
d. Click on the Adobe Acrobat symbol to view, print, or download the document, or check the box of the desired document and delivery method (fax or e-mail)</td></tr>
<tr><td>By automated telephone service from any country</td><td>Use “To Obtain Documents on Demand” on page D-5.</td></tr>
<tr><td>By telephone in the United States</td><td>Dial 1-800-327-3002, then press 1</td></tr>
<tr><td>By telephone from Canada</td><td><table><tr><th>To order in...</th><th>Then dial 1-800-668-6913 and...</th></tr><tr><td>English</td><td>Press 1, then 2, then 1 again</td></tr><tr><td>French</td><td>Press 2, then 2, then 1</td></tr></table></td></tr>
<tr><td>By telephone from any other country</td><td>See “Technical Support” on page D-1.</td></tr>
</table>

For chemicals not manufactured or distributed by Applied Biosystems, call the chemical manufacturer.

Instrument Safety Labels

Safety labels are located on the instrument. Each Safety label consists of a:

- Signal Word panel, which implies a particular level of observation or action (*e.g.,* CAUTION or WARNING). If a safety label encompasses multiple hazards, the Signal Word corresponding to the greatest hazard is used.
- Message panel, which explains the hazard and any user action required.
- Safety Alert symbol, which indicates a potential personal safety hazard. See the Procise/Procise cLC *Site Preparation and Safety Guide* for an explanation of all Safety Alert symbols provided in multiple languages.

About Waste Profiles

A waste profile was provided with this instrument and is contained in the *Procise and Procise cLC Site Preparation and Safety Guide.* Waste profiles list the percentage compositions of the reagents within the waste stream at installation and the waste stream during a typical user application. These profiles assist users in planning for instrument waste handling and disposal, which must be in accordance with local, state/provincial, or national regulations. Read the waste profiles and all applicable MSDSs before handling or disposing of waste.

IMPORTANT Waste profiles are not a substitute for MSDS information.

Before Operating the Instrument

Ensure that everyone involved with the operation of the instrument has:

- Received instruction in general safety practices for laboratories
- Received instruction in specific safety practices for the instrument
- Read and understood all related MSDSs

⚠ CAUTION Avoid using this instrument in a manner not specified by Applied Biosystems. Although the instrument has been designed to protect the user, this protection can be impaired if the instrument is used improperly.

Safe and Efficient Computer Use

Operating the computer correctly prevents stress-producing effects such as fatigue, pain, and strain.

To minimize these effects on your back, legs, eyes, and upper extremities (neck, shoulder, arms, wrists, hands and fingers), design your workstation to promote neutral or relaxed working positions. This includes working in an environment where heating, air conditioning, ventilation, and lighting are set correctly. See the guidelines below.

⚠ CAUTION **MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD**. These hazards are caused by the following potential risk factors which include, but are not limited to, repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

- Use a seating position that provides the optimum combination of comfort, accessibility to the keyboard, and freedom from fatigue-causing stresses and pressures.
  - The bulk of the person's weight should be supported by the buttocks, not the thighs.
  - Feet should be flat on the floor, and the weight of the legs should be supported by the floor, not the thighs.
  - Lumbar support should be provided to maintain the proper concave curve of the spine.

- Place the keyboard on a surface that provides:
  - The proper height to position the forearms horizontally and upper arms vertically.
  - Support for the forearms and hands to avoid muscle fatigue in the upper arms.
- Position the viewing screen to the height that allows normal body and head posture. This height depends upon the physical proportions of the user.
- Adjust vision factors to optimize comfort and efficiency by:
  - Adjusting screen variables, such as brightness, contrast, and color, to suit personal preferences and ambient lighting.
  - Positioning the screen to minimize reflections from ambient light sources.
  - Positioning the screen at a distance that takes into account user variables such as nearsightedness, farsightedness, astigmatism, and the effects of corrective lenses.
- When considering the user's distance from the screen, the following are useful guidelines:
  - The distance from the user's eyes to the viewing screen should be approximately the same as the distance from the user's eyes to the keyboard.
  - For most people, the reading distance that is the most comfortable is approximately 20 inches.
  - The workstation surface should have a minimum depth of 36 inches to accommodate distance adjustment.
  - Adjust the screen angle to minimize reflection and glare, and avoid highly reflective surfaces for the workstation.
- Use a well-designed copy holder, adjustable horizontally and vertically, that allows referenced hard-copy material to be placed at the same viewing distance as the screen and keyboard.
- Keep wires and cables out of the way of users and passersby.
- Choose a workstation that has a surface large enough for other tasks and that provides sufficient legroom for adequate movement.

Reagents and Solvents Used on the System

Table of Chemical Storage Conditions

All reagents and solvents supplied by Applied Biosystems are highly purified and sequencer-tested to ensure optimal performance. A list of reagents and solvents used with the standard sequencer cycles is given in Table 1-1.

Table 1-1 Chemicals Used on the Procise Protein Sequencer

Bottle Position	Chemical Contents	Part Number	Storage Conditions
R1	R1, 5% phenylisothiocyanate (PITC) in heptane	400208	–20 °C [a]
R2	R2B, N-methylpiperidine/water/methanol (MeOH)	401535	4 °C [a]
R3	R3, Trifluoroacetic acid (TFA), neat	400003	RT [b]
R4	R4A, 25% TFA in water, with 0.01% dithiothreitol (DTT)	400028	4 °C [a]
R5	PTH standard plus R5, acetonitrile, with 0.001% DTT	400879 400315	RT [b]
S1	S1, n-heptane (not used)	400079	RT [b]
S2	S2B, ethyl acetate	400854	RT [b]
S3	S3, n-butyl chloride	400008	RT [b]
S4	S4B, 20% acetonitrile in water	400314	RT [b]
X1	50% methanol/water		
X2	Acetonitrile		
X3	Not used		
—	20 Amino Acid PTH standard	400879	–20 °C [a]
—	Biobrene Plus	400385	4 °C [a]
—	ß-lactoglobulin	400979	4 °C [a]

a. Allow these items to reach room temperature before opening. When these bottles are opened while still cold, water can condense inside. Check bottle caps for tightness after placing these bottles at either 4 °C (2–8 °C) or –20 °C (–15 to –20 °C).

b. RT (Room Temperature) = 15–20 °C in a dark, dry place.

Supporting Chemicals

Supporting solvents and chemicals needed to run the system are listed below.

Chemicals	Part Number	Storage Conditions
Solvent A3, 3.5% THF Tetrahydrofuran in Water, 1 L (Procise)	401464	RT
Solvent B2, 12% Isopropanol and Acetonitrile, 1 L (Procise)	401570	RT
Solvent B (not used),100% acetonitrile, 1 L	400313	RT
Acetone (HPLC grade)		RT
MeOH (Methanol)	400470	RT
Premix Buffer Concentrate	401446	4 °C

Chemical Warnings Table

Table 1-2 Warnings on Chemicals Used in the Procise System

Chemical	Chemical Hazard
A3 (3.5% tetrahydrofuran in water)	⚠ **WARNING** **CHEMICAL HAZARD. A3 (tetrahydrofuran in water)** is a flammable liquid and vapor. It may be harmful if swallowed. Exposure may cause eye and respiratory tract irritation, central nervous system depression, and liver and kidney damage. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
Acetone	⚠ **WARNING** **CHEMICAL HAZARD. Acetone** is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin. It may cause central nervous system effects such as drowsiness, dizziness, headache, etc. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
Acetonitrile (Also in R5, S4B, B, B2)	⚠ **WARNING** **CHEMICAL HAZARD. Acetonitrile** is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation, central nervous system depression, and heart, liver, and kidney damage. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
Argon	⚠ **CAUTION** **CHEMICAL HAZARD. Argon** is a nonflammable high-pressure gas. Released argon gas reduces the oxygen available for breathing. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
B2 (12% isopropanol and acetonitrile)	⚠ **WARNING** **CHEMICAL HAZARD. B2 (12% isopropanol and acetonitrile)** is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation. Prolonged or repeated contact may dry skin. Exposure may cause central nervous system depression, and heart, liver, and kidney damage. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
Biobrene Plus	⚠ **CAUTION** **CHEMICAL HAZARD. Biobrene Plus** may cause eye, skin, and respiratory tract irritation. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
Dithiothreitol (DTT) (In R4A, R5)	⚠ **CAUTION** **CHEMICAL HAZARD**. **Dithiothreitol (DTT)** may cause eye, skin, and respiratory tract irritation, central nervous system depression, and damage to the kidneys. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
Isopropanol	⚠ **WARNING** **CHEMICAL HAZARD. Isopropyl alcohol** can be harmful if inhaled, ingested, or absorbed through the skin. It can cause CNS depression, and be irritating to the eyes, skin, and mucous membranes.

Table 1-2 Warnings on Chemicals Used in the Procise System *(continued)*

Chemical	Chemical Hazard
Methanol (Also in R2B)	⚠ **WARNING** **CHEMICAL HAZARD. Methanol** is a flammable liquid and vapor. Exposure may cause eye, skin, and respiratory tract irritation, and central nervous system depression and blindness. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
Piperidine (hexahydropyridine)	⚠ **WARNING** **CHEMICAL HAZARD. Piperidine** (Hexahydropyridine) can cause severe burns to the skin, eyes, and respiratory tract. It is a potential reproductive toxicant, potential carcinogen, that can affect the heart, liver, and kidney. It is flammable as a gas or as a liquid. Avoid all skin contact with and inhalation of piperidine.
PMTC	⚠ **WARNING** **CHEMICAL HAZARD. PMTC** (in acetonitrile) is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation, central nervous system depression, and heart, liver, and kidney damage. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
Premix Buffer Concentrate	⚠ **DANGER** **CHEMICAL HAZARD. Premix Buffer** Concentrate causes burns to the eyes, skin, and respiratory tract. It is a combustible liquid and vapor. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
R1 (5% phenylisothiocyanate in n-heptane)	⚠ **DANGER** **CHEMICAL HAZARD. R1 (5% phenylisothiocyanate in n-heptane)** causes burns to the eyes, skin, and respiratory tract. It is a flammable liquid and vapor. Exposure may cause an allergic skin reaction and central nervous system effects such as drowsiness, dizziness, headache, etc., and irregular heartbeats. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
R2B (n-methylpiperidine in methanol and water)	⚠ **WARNING** **CHEMICAL HAZARD. R2B (n-methylpiperidine in methanol and water)** is a flammable liquid and vapor. Exposure may cause eye, skin, and respiratory tract irritation, and central nervous system depression and blindness. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
R3 (trifluoroacetic acid)	⚠ **DANGER** **CHEMICAL HAZARD. R3 (trifluoroacetic acid)** causes severe burns to the eyes, skin, and respiratory tract. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
R4 (25% trifluoroacetic acid in water)	⚠ **DANGER** **CHEMICAL HAZARD. R4 (25% trifluoroacetic acid in water)** causes severe burns to the eyes, skin, and respiratory tract. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Table 1-2 Warnings on Chemicals Used in the Procise System *(continued)*

Chemical	Chemical Hazard
R5 (0.001% DTT in acetonitrile)	⚠ WARNING **CHEMICAL HAZARD. R5 (0.001% DTT in acetonitrile)** is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation, central nervous system depression, and heart, liver, and kidney damage. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
Sodium phosphate	⚠ CAUTION **CHEMICAL HAZARD. Sodium phosphate** may cause eye, skin, and upper respiratory tract irritation. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
S1 (n-heptane)	⚠ WARNING **CHEMICAL HAZARD. S1 (n-heptane)** is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation. Prolonged or repeated contact may dry skin. It may cause central nervous system effects such as drowsiness, dizziness, headache, etc., and irregular heartbeats. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
S2B (ethyl acetate)	⚠ WARNING **CHEMICAL HAZARD. S2B (ethyl acetate)** is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation. Prolonged or repeated contact may dry skin. It may cause central nervous system effects such as drowsiness, dizziness, headache, etc. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
S3 (n-butyl chloride)	⚠ WARNING **CHEMICAL HAZARD. S3 (n-butyl chloride)** is a flammable liquid and vapor. Exposure may cause central nervous system effects such as drowsiness, dizziness, headache, etc. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
S4B (20% acetonitrile in water)	⚠ WARNING **CHEMICAL HAZARD. S4B (20% acetonitrile in water)** is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation, central nervous system depression, and heart, liver, and kidney damage. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
Sodium dodecyl sulfate (SDS)	⚠ WARNING **CHEMICAL HAZARD. Sodium dodecyl sulfate (SDS**) may cause an allergic respiratory reaction. It is harmful if inhaled, swallowed, or absorbed through the skin. Exposure causes eye, skin, and respiratory tract irritation. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Sequence Analysis Chemistry 2

Overview

About This Chapter

This chapter describes the Edman chemistry used by the Procise® Protein Sequencing System. A description of the chemicals, their stability and storage requirements is included here. The analysis of PTH-amino acids by reversed-phase HPLC is also outlined.

In This Chapter

This chapter contains the following topics:

Introduction

About the Chemical Process

The Procise Protein Sequencer automates a chemical process that removes and analyzes amino acid residues from protein and peptide chains of various types and lengths. This process is derived from the technique developed by Pehr Edman for the sequential degradation of proteins and peptides. Edman degradation relies on the specific reactivity of a protein's N-terminal amino acid and the selective removal of the derivatized amino acid from the protein, while leaving the rest of the molecule intact. Each cycle of the degradation sequentially removes an amino acid from the amino terminal end of the protein or peptide sample. This cyclic process provides the primary structure.

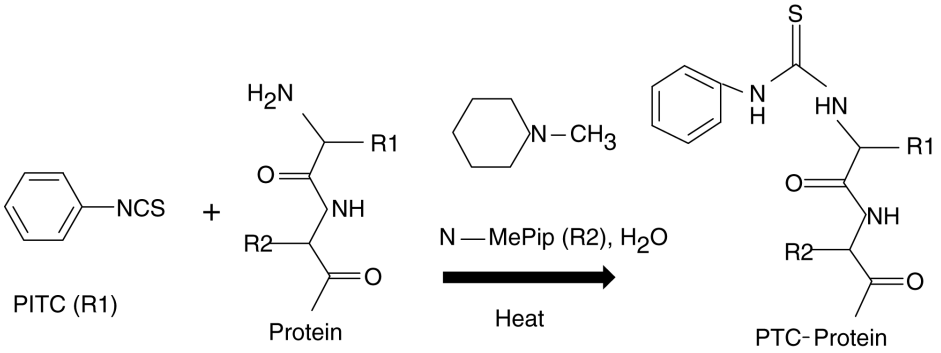
Two Sequencing Methods

The Procise Protein Sequencer offers a variety of sequencing methods. One method is optimized for protein or peptide samples applied to a glass-fiber filter previously treated with BioBrene™ Plus, and then cycled through a conditioning process of one or more repetitions of the Edman chemistry. The conditioning process reduces contaminants and improves the efficiency of subsequent sample sequencing.

Another method is optimized for samples bound to polyvinylidine difluoride (PVDF) membrane. A specially designed reaction chamber, the Blott™ cartridge, is used for these membrane-bound samples. The Blott cartridge is a vertical cross-flow reactor that facilitates optimization of the cartridge chemistry.

Edman Degradation

The Coupling Reaction



Coupling with R1

R1 (5% PITC in n-heptane) is a sweet-smelling, volatile liquid. In the presence of base, PITC (R1) reacts quantitatively with the free amino groups of a peptide or protein (including side-chain amino groups) to form the phenylthiocarbamyl-derivative (PTC-derivative). In standard sequencer cycles, R1 is loaded and delivered to the cartridge on a stream of argon to wet the glass fiber filter. Three separate R1 deliveries are alternated with vapor deliveries of base (R2B). These repeated R1 deliveries increase the reagent-protein contact and improve the reaction yield. After each R1 delivery, argon removes the non-reactive solvent, n-heptane.

Coupling with R2B

R2B (N-methylpiperidine/water/methanol) is a strongly basic amine, identifiable in higher concentrations by its strong “ammonia” odor. R2B provides the basic environment necessary for PITC to react with the amino terminus of the sample. In the standard cycles, R2B is delivered to the filter cartridge between three R1 deliveries and argon drying steps.

IMPORTANT **Oxidation**: Once coupling has occurred, the PTC-peptide or PTC-protein is susceptible to oxidative de-sulfurization. Replacement of sulfur with oxygen inhibits cyclization and cleavage, thus reducing yields. For this reason, all solvents and reagents are stored and maintained under argon and should be purged with argon whenever replaced.

Post-Coupling Extraction

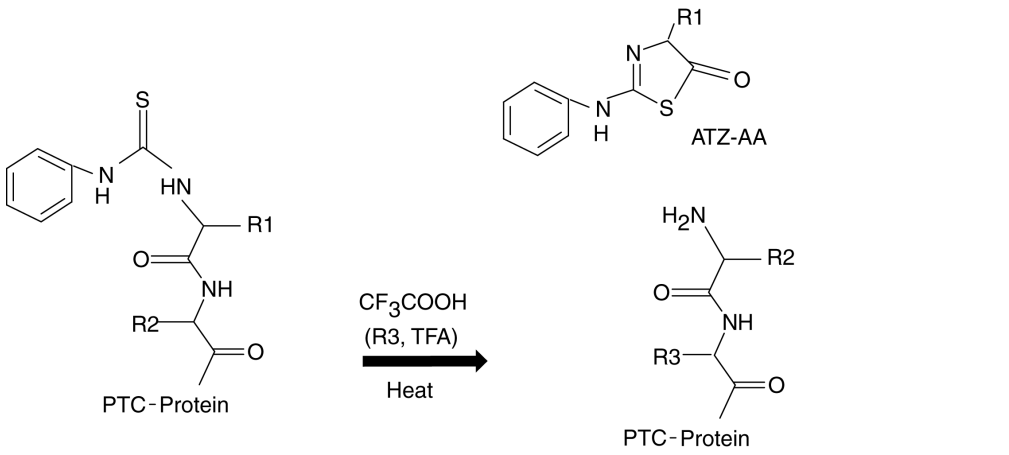
Solvents are delivered through the filter to extract unwanted materials that could hinder later reactions or form byproducts which could interfere with the chromatographic analysis of PTH amino acids. The solvents help remove the following:

- PITC (R1) byproducts, generally thioureas and ureas
- Residual PITC to minimize additional thiourea formation
- Residual N-methylpiperidine (R2B) to avoid salt formation during TFA (R3) delivery
- Residual water (for which BioBrene Plus has an affinity) to minimize acid-catalyzed hydrolysis during cleavage

Extracting with Solvents S2B and S3

S2B (ethyl acetate) and S3 (butyl chloride) remove the majority of these unwanted materials. The amounts of S2B and S3 delivered can be increased beyond that listed in the standard sequencer cycles. While this minimizes unwanted artifact levels, excessive S2B delivery may cause extractive loss of some small peptides. Chapter 4, “Creating Functions, Cycles, and Methods,” describes how to create cycles and methods.

Cleavage Diagram



Cleaving with R3

R3 (TFA) is a highly volatile, strong organic acid. R3 cleaves the PITC-coupled amino acid residue from the amino terminus of the protein, thereby producing the anilinothiazolinone (ATZ) derivative of the amino acid. The remaining protein is left with a new amino terminus which will be coupled with PITC in the next cycle.

ATZ Transfer

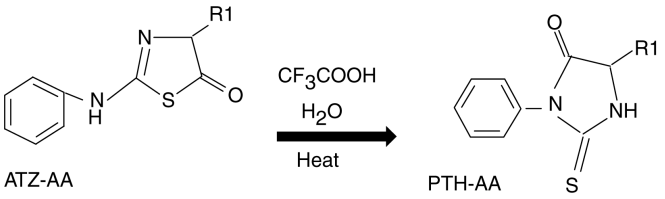
S3 (butyl chloride) and/or S2B (ethyl acetate)

S3 (butyl chloride) and/or S2B (ethyl acetate) are used to extract the cleaved ATZ amino acid from the protein and transfer it from the filter cartridge to the conversion flask. Both S3 and S2B are used for extraction in the glass fiber filter cycles and S2B is used for extraction in the Blot (PVDF membrane). One delivery each of S3 and S2B, or three S2B deliveries transfer the ATZ amino acid to the conversion flask. The last portion of this solution is transferred to the flask with argon.

A small amount of S4B (20% acetonitrile/water) is delivered to the flask just before the extraction solution reaches it. This S4B delivery minimizes exposure of the labile ATZ amino acids to the hot walls of the flask.

After the extractions are completed, an R2B delivery to waste and a short argon dry precede an additional S2B delivery through the cartridge to the waste bottle. These deliveries are essential to rinse remaining R3 from the cartridge and facilitate neutralization of the sample prior to the next sequencing cycle.

PTH Conversion Diagram



Converting with R4A

The transferred extraction solution (S3 and/or S2B + ATZ amino acid), along with most of the initial delivery of S4B, is evaporated from the conversion flask. An aliquot of R4A (25% TFA in water – aqueous TFA) is then delivered to the flask to convert the ATZ-AAs to the more stable PTH-AAs. The presence of dithiothreitol in the R4A minimizes oxidation of the ATZ amino acids. In standard sequencer cycles, conversion occurs for approximately nine minutes at 64 °C. These conditions are sufficient to give virtually complete conversion of all amino acids except glycine (about 15% of derivatized glycine remains in the intermediate PTC-glycine form).

R5

R5 (acetonitrile) is used to introduce an aliquot of the PTH standard mix to the flask for subsequent HPLC analysis.

Solubilization and Injection of PTH-AAs with S4B

After conversion is complete, R4A is evaporated from the flask, leaving behind the dried residue of the PTH-AAs. The residue is dissolved in S4B (20% aqueous acetonitrile) and transferred to the HPLC system.

In standard sequencer cycles, two metered volumes of S4B are delivered to the flask, mixed, and allowed to incubate to ensure complete dissolution. The solution is then transferred to the HPLC system. Subsequent S4B deliveries are made to pick up any residual PTH amino acid and to rinse the lines between the conversion flask and the injector. In preparation for the next conversion, S4B is then delivered to fill the flask, mixed by argon bubbling, and emptied to waste.

Separation and Analysis of PTH-AAs

The HPLC pump forces the sample mixture and mobile phase through the column under pressure. The various components of the sample having different relative affinities for the stationary phase inside the column will exit the column at different times corresponding to different solvent strengths. The pump flow rate, the solvent composition, and the oven temperature determine the speed and order of elution of the PTH-AAs in the sample.

The mobile phase/amino acid mixture travels from the end of the column into a detector flowcell. The detector measures the UV light absorbance of the column effluent and the analog signal is output to external recording devices.

Valves, Functions, Cycles and Methods 3

Overview

About This Chapter This chapter defines the components that make up a sequencing run.

In This Chapter This chapter contains the following topics:

Components of a Run

Table of Components

Definitions of the components that constitute a run are described below. Each component is discussed in this chapter.

Run Component	Definition
Valve	A valve is a mechanical device which opens to provide a flow path for the transfer of gas, solvent, or reagent. The Procise® Protein Sequencer has three types of valves that perform one of the following functions: ♦ Deliver liquids, vapors or gas ♦ Deliver only gas ♦ Vent the chemical bottles
Function	Functions activate a valve or a set of valves to deliver a chemical, activate or deactivate a relay, define or increment a setpoint, or tell the pump to start or stop. Customized functions can be created by the user.
Step	A function, once incorporated into a cycle, becomes a step of that cycle. This function may either have a fixed or a global time associated with it.
Cycle	A cycle is a series of steps, in an order specified by the standard chemistry or by the user, that accomplishes a specific chemical process (in the reaction cartridge and/or the conversion flask). Standard cycles (those supplied by Applied Biosystems) are permanently stored in memory. The user can also create new cycles or edit existing cycles.
Method	A method consists of the cycles (repetitions of the same cycle or different cycles) needed to sequence a peptide/protein sample. A typical method may start with one cycle, then perform many repetitions of another cycle. Methods also include the appropriate starting temperatures for the cartridge, flask, and column as well as the gradient to be run by the HPLC. Standard methods (those supplied by Applied Biosystems) are permanently stored in memory. The user can also create new methods or edit existing methods.
Gradient	A gradient is a pump program that defines flow rate and solvent composition changes over time.

See Chapter 4, “Creating Functions, Cycles, and Methods,” for more information on functions, steps, cycles and methods.

Valves

About Valves

Gas and chemical deliveries are controlled by valves that open to create delivery pathways to a particular destination, such as the reaction cartridge. Each valve is assigned a number. The valve diagram (Figure 3-1 on page 3-4) displays the placement of each valve. Valves are opened (activated) and closed (deactivated) electrically.

Delivery Valves Table

Delivery valves are built together into valve blocks. The chemical delivery system is managed by seven valve blocks interconnected with Teflon tubing.

Types of Valve Blocks

Valve Block	Control Function
Cartridge reagent block	Controls delivery of reagents [R1, R2, X1(liquid and gas), and X3] to the cartridge input block and to waste.
Cartridge solvent block	Controls delivery of one reagent (R3), solvents (S2, S3, and S1) and argon to the cartridge reagent block, cartridge input block, cartridge output block, and to waste.
Cartridge input block	Controls transfer and metering of reagents, solvents, and argon from the cartridge reagent block and cartridge solvent block into or out of the active cartridge, and to waste.
Cartridge output block	Controls transfer of reagents, solvents and argon from the cartridge reagent block and cartridge solvent block into or out of the active cartridge and to waste.
Flask reagent block	Controls delivery and metering (small loop) of reagents [R4, R5, X2 (liquid), and X3], solvent (S4), and argon to the flask input block.
Flask input block	Controls delivery [X2 (gas)], transfer, and metering (large loop) of reagents, solvents, and argon from the flask reagent block to the conversion flask and to waste.
Flask output block	Controls delivery of argon to the conversion flask for bubbling and for flushing the sample loop, and transfer of the contents of the conversion flask to the sample loop and to waste.

Designed for Delivery

The design of the valve blocks minimizes any holdup volume following chemical delivery. Delivery lines feed into each valve block and connect to the common pathway (manifold) inside the block through a manifold inlet line and a solenoid-controlled valve.

Passage between a manifold inlet line and the manifold occurs only when the appropriate valve is activated. The manifold zigzags through the valve block and passes other closed valves that are unaffected by the flow. The direction of the flow is determined by the pressures on either side of the pathway. See Figure 3-1 for the valve diagram on reagent, solvent, and gas flow.

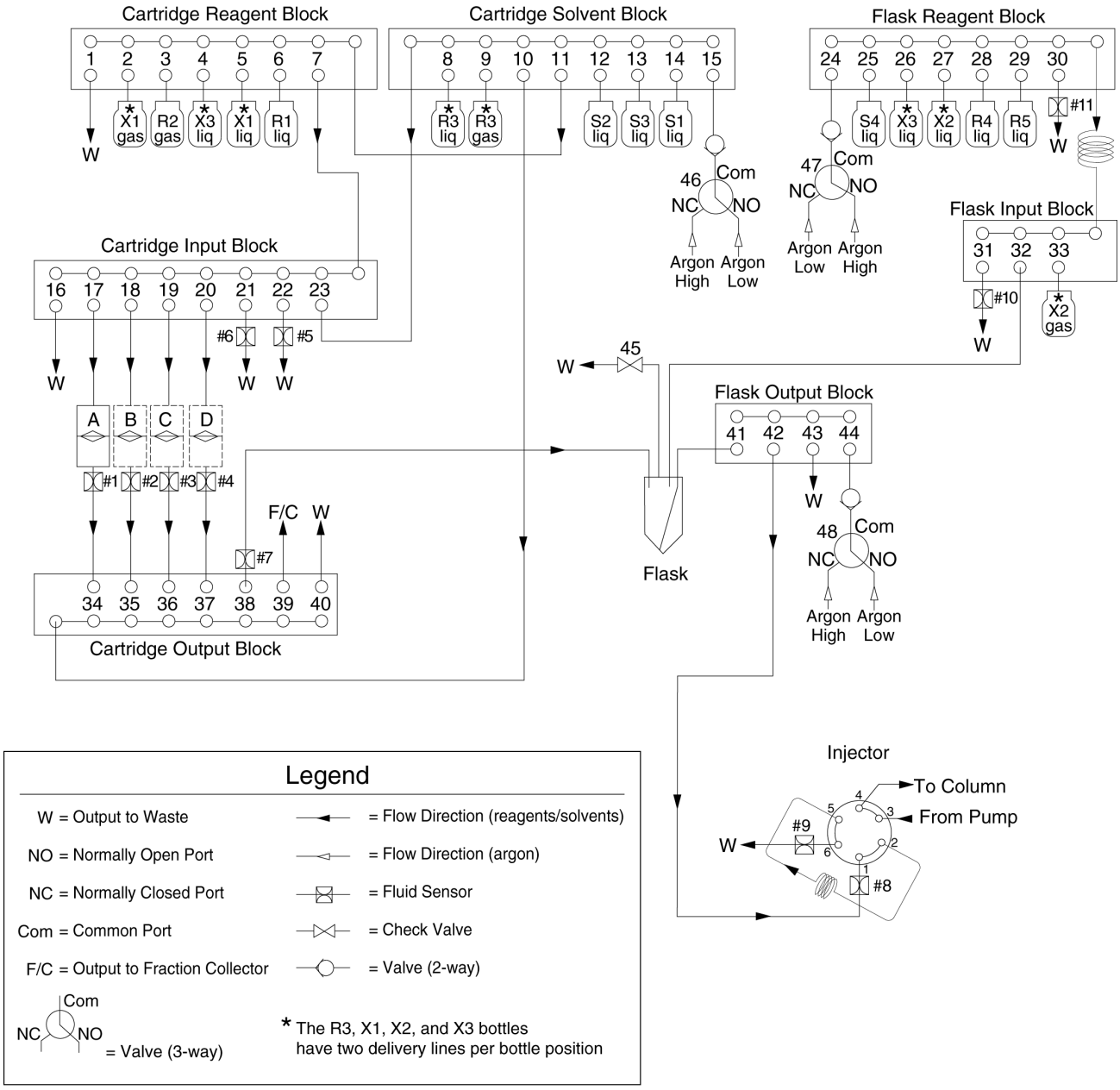


Figure 3-1 Procise Valve Diagram

Bottle Vent Valves

The bottle vent valves control argon flow for bottle pressurization and flushing. There are 12 vent valves in the Procise Protein Sequencer, one for each chemical bottle. During chemical delivery, these valves remain closed. During venting, flushing, or backflushing, these valves are open to allow argon in the bottle headspace to flow to waste. The vent valves are also activated by the pressure control system in order to maintain proper bottle pressurization.

Three-Way Valves

Three-way valves are used exclusively for argon delivery. Three of these valves are used in the protein sequencer. The three-way valves provide two different argon pressures from the same manifold inlet line. Three-way valves control argon input to valve positions 15, 24, and 44, and provide high and low pressure settings.

Table 3-1 Standard Pressures with Three-Way Valves

Valve Status	Function(s)	Pressure
Valve 46 off	Cart dry	3.5 psi
Valve 46 on	All cart block flushing	Internal manual regulator pressure
Valve 47 off	Flask dry all flask flushing	3.0 psi
Valve 47 on	Load injector	1.0 psi
Valve 48 off	Flask bubble, low pressure: sample loop flushing	1.8 psi
Valve 48 on	High pressure sample loop flushing	Internal manual regulator pressure

Functions

About Functions

For the instrument to perform any task, such as delivering a reagent, valves must be activated. To simplify programming and operation, valves are grouped into functions. Each function is numbered and given a name that describes its purpose. Table 3-2 provides an outline of the function numbering and naming format.

Function Numbering Format

The complete function list can be found in Appendix A, “Function Listing,” or from the Functions view in the Procise software.The function numbering system is:

- Standard functions are numbered 1–400.
- User functions are numbered 401–450.

Table 3-2 Function Numbering Format

Function Number	Function
1–150	Cartridge
151–250	Flask/HPLC
251–259	Transfer
260–360	Test/Procedure
401–450	User

Valve Controlling Functions

These functions activate a valve or set of valves simultaneously to deliver a chemical or gas. These functions are also called time-dependent functions because the valves are activated (opened) for a fixed time as prescribed in a cycle or by the user in manual control mode, and then deactivated at the end of the step. The majority of functions operate in this manner.

The flowpath created by activating a valve-controlling function can be traced using the valve diagram (Figure 3-1 on page 3-4).

Sensor Functions

Sensor functions are specialized valve-controlling functions. These functions are controlled by the target sensor, rather than by a specific time setting. When a function is activated, the sensor begins “looking” for fluid. When fluid is detected by the sensor, the reagent or solvent delivery valve is turned off. The time for the function continues to count down to 0, and the next step begins.

For a sensor function to perform correctly, enough time must be allotted in the step for fluid to reach the sensor. If fluid does not reach the sensor within the allotted time, an error will be reported in the event log and the sequencer may be paused.

Cycle-Synchronizing Functions

A group of functions are used to provide proper synchronization of the cartridge and flask cycles during sequencing. Every cycle must have Begin (Function 258) and End (Function 259) steps in order to be valid. The flask or the cartridge cycle will wait at the Begin step, if necessary, in order to keep the cycles synchronized. The cycle timer will count up (increment) during this step if a cycle is synchronizing. At the appropriate time, the cycle will automatically proceed to the next step.

The timing for transfer from cartridge to flask is defined by a set of steps in the cartridge cycle and one step in the flask cycle. A Ready to Receive (Function 228) step in the flask cycle indicates that the flask is ready to accept sample transfer from

the cartridge. The flask will wait at this step, with the cycle timer incrementing, until the transfer is complete.

The beginning and end of the transfer from the cartridge to the flask are defined in the cartridge cycle respectively by, the steps Ready Transfer to Flask (Function 127) and Transfer Complete (Function 128). Synchronization is set up in such a way that the Ready to Receive step in a flask cycle occurs 5 seconds before the Ready to Transfer step in the cartridge cycle.

Other System Functions

Table 3-3 Necessary Cartridge Functions

Function Name	Number	Description
Set Cart Temperature	142	Used to adjust the cartridge temperature at a fixed time during a cycle. Acceptable temperature range: ambient to 70 °C.

Table 3-4 Necessary Flask Functions

Function Name	Number	Description
Load Position	226	Switches the sample loop out of the HPLC flow path. During a flask cycle, this function must precede the Load Injector step in order for the sample loop to be flushed and the sample in the flask to be transferred into the sample loop.
Set Flask Temperature	230	Used to adjust the flask temperature at a fixed time during a cycle. Acceptable temperature range: ambient to 78 °C.
Prepare Pump	227	Downloads gradient information from the Procise software to the pump. After downloading is complete (30–60 sec.), the pump will start, pressurize, and run at the initial gradient conditions.
Stop Pump	231	Stops any pump activity.
Start Gradient	232	Used to start the gradient in cases where there will be no sample injected.
Inject Position	223	Switches the sample loop into the HPLC flow path. Not necessary when using the Sample Loop Load sensor. When the sensor detects fluid, the Rheodyne valve is automatically activated.
Set Column Temperature	229	Used to adjust the column temperature at a fixed time during the flask cycle. Acceptable temperature range: ambient to 70 °C.

Table 3-5 Common Functions

Function Name	Number	Description
Begin	258	This function must be the first step of all cycles, tests, and procedures.
End	259	This function must be the last step of all cycles, tests, and procedures.
Wait	257	This function is used to keep the cycle time running for a particular step in a cycle while all the valves are closed.

User Functions

User functions can be created and named for specialized needs or applications. Fifty potential functions are available. An explanation of creating user functions can be found in “Creating User-Defined Functions” on page 4-2.

Cycles

About Cycles

To perform complex chemical tasks, groups of functions are organized together into cycles, defined here in Table 3-6. Once incorporated into a cycle, individual functions are referred to as steps of that cycle and will be activated for a specified period of time during that cycle.

Table 3-6 Defining the Cycle Types

Cycle Type	Definition
Cartridge cycles	Chemical processes occurring in the glass reaction cartridge blocks are referred to as cartridge cycles.
Flask cycle	Processes that take place in the conversion flask are referred to as flask cycles.
Standard cycles	The permanent cycles provided with the protein sequencer are referred to as standard cycles.

Note These cycles can not be deleted or modified, but can be used as templates for creating new cycles after the cycle has been stored under a new name. The specifics of creating new cycles are described in “Creating Cycles” on page 4-4.

Standard Cartridge Cycles

There are nine standard cartridge chemistry cycles. A complete cartridge cycle listing can be found in Appendix B, “Cycle, Method, and Gradient Listings.”

Table 3-7 Standard Cartridge Cycles

Cycle	Description
Cart Precycle	Prepares a polybrene-treated glass fiber disk for sequencing by running abbreviated coupling and repetitive cleavage reactions.
Cart Begin	Prepares sample for pulsed liquid sequencing by delivering an aliquot of TFA to denature the sample followed by coupling with PITC.
Cart Begin Gas-phase	Gas-phase prepares sample for sequencing by delivering TFA vapor to denature the sample followed by coupling with PITC.
Cart Pulsed-liquid	Edman chemistry cycle for sequencing samples on polybrene-coated glass fiber disks. Delivers an aliquot of liquid TFA for cleavage. (Three ATZ extractions: two with butyl chloride, one with ethyl acetate.)
Cart Gas-phase	Edman chemistry cycle for sequencing samples on polybrene-coated glass fiber disks. Delivers TFA vapor for cleavage. (Three ATZ extractions: two with butyl chloride, one with ethyl acetate.)
Cart PL PVDF Protein	Edman chemistry cycle for sequencing protein samples on PVDF membrane. Delivers an aliquot of liquid TFA for cleavage. Includes pre-wetting of the membrane with methanol/water (X1 bottle) prior to coupling and an additional extraction of the membrane after cleavage. (Three ATZ extractions, two with butyl chloride, one with ethyl acetate.)
Cart PL PVDF Peptide	Edman chemistry cycle for sequencing peptide samples on PVDF membrane. Delivers an aliquot of liquid TFA for cleavage. (Three ATZ extractions, two with butyl chloride, one with ethyl acetate.)
Cart GP PVDF Protein	Edman chemistry cycle for sequencing protein samples on PVDF membrane. Delivers TFA vapor for cleavage. Includes pre-wetting of the membrane (with methanol/water from X1 bottle) prior to coupling and an additional extraction of the membrane after cleavage. (Three ATZ extractions, two with butyl chloride, one with ethyl acetate.)

Table 3-7 Standard Cartridge Cycles *(continued)*

Cycle	Description
Cart GP PVDF Peptide	Edman chemistry cycle for sequencing peptide samples on PVDF membrane. Delivers TFA vapor for cleavage. (Three ATZ extractions, two with butyl chloride, one with ethyl acetate.)

Standard Flask Cycles

There are five standard flask chemistry cycles. A complete flask cycle listing can be found in “Flask Cycle List” on page B-25.

Table 3-8 Standard Flask Cycles

Cycle	Description
Flask Blank	Performs conversion chemistry and reconstitution without any sample or standard. Starts HPLC and transfers flask contents to the sample loop for analysis.
Flask Standard	Performs conversion chemistry and reconstitution with PTH-amino acid standard mixture in the flask. Starts HPLC and transfers flask contents to the sample loop for analysis.
Flask Normal	For converting the ATZ-amino acid transferred from the cartridge to a PTH-amino acid. Starts HPLC and transfers flask contents to the sample loop for analysis.
Flask Prep Pump	Downloads instructions to the HPLC pump to run at 50% B while the flask blank is being prepared.
Run Gradient	Used in troubleshooting chromatography problems to isolate the HPLC. Downloads gradient to HPLC, equilibrates column, and runs gradient. No injection occurs.

Methods

About Methods

A method combines a number of cartridge cycles, flask cycles, HPLC gradients and operating temperature settings in the order necessary to perform a specific task such as the analysis of a protein sample. There are sequence methods for preparing glass fiber filters prior to sample loading, methods specific to the sample substrate and the type of cleavage employed and methods for testing or optimizing the sequencer performance.

Table 3-9 Standard Methods

Method	Used to...
Filter Precycle	prepare a glass fiber disk that has been treated with Biobrene Plus solution prior to applying the protein or peptide sample. The Filter Precycle method includes: ♦ Two filter conditioning cycles ♦ A PTH-amino acid standard cycle ♦ A sequencing cycle that can be repeated a user-selectable number of times allowing evaluation of the chemical and amino acid background level This method requires at least 2.5 hours for completion.
Fast Precycle	prepare a glass fiber disk that has been treated with Biobrene Plus solution prior to applying the protein or peptide sample. The Fast Precycle method includes: ♦ Two filter conditioning cycles ♦ A PTH-amino acid standard cycle This method can be completed in as little as 1 hour, but does not allow evaluation of the chemical and amino acid background level prior to sample loading.
Pulsed-liquid	sequence a protein or peptide sample that has been applied to a precyled glass fiber disk. The Pulsed-liquid method includes: ♦ A begin cycle to prepare the sample for sequencing ♦ A blank cycle ♦ A PTH-amino acid standard cycle ♦ Sequencing cycles The Pulsed-liquid method delivers a small volume of liquid TFA to the cartridge for cleavage of the ATZ-amino acid.
Gas-phase	sequence a protein or peptide sample applied to a precycled glass fiber disk. The Gas-phase method includes: ♦ A begin cycle to prepare the sample for sequencing ♦ A blank cycle ♦ A PTH-amino acid standard cycle ♦ Sequencing cycles The Gas-phase method delivers TFA vapor to the cartridge for cleavage of the ATZ-amino acid.

Table 3-9 Standard Methods *(continued)*

Method	Used to...
PL PVDF Protein	sequence a protein sample that has been applied to PVDF membrane by electroblotting or using a ProSorb™ device. The PL PVDF Protein method includes: ♦ A begin cycle to prepare the sample for sequencing ♦ A blank cycle ♦ A PTH-amino acid standard cycle ♦ Sequencing cycles The PL PVDF Protein method delivers a small volume of liquid TFA to the cartridge for cleavage of the ATZ-amino acid and includes a delivery of 50% methanol from the X1 bottle to the cartridge during coupling to improve the repetitive yield.
GP PVDF Protein	sequence a protein sample that has been applied to PVDF membrane by electroblotting or using a ProSorb device. The GP PVDF Protein method includes: ♦ A begin cycle to prepare the sample for sequencing ♦ A blank cycle ♦ A PTH-amino acid standard cycle ♦ Sequencing cycles The GP PVDF Protein method delivers TFA vapor to the cartridge for cleavage of the ATZ-amino acid and includes a delivery of 50% methanol from the X1 bottle to the cartridge during coupling to improve the repetitive yield.
PL PVDF Peptide	sequence a peptide sample that has been applied to PVDF membrane using a ProSorb device. The PL PVDF Peptide method includes: ♦ A begin cycle to prepare the sample for sequencing ♦ A blank cycle ♦ A PTH-amino acid standard cycle ♦ Sequencing cycles The PL PVDF Peptide method delivers a small volume of liquid TFA to the cartridge for cleavage of the ATZ-amino acid. A few microliters of BiobrenePlus solution diluted 1:10 with methanol should be applied to the sample membrane before sequencing.
GP PVDF Peptide	sequence a peptide sample that has been applied to PVDF membrane using a ProSorb device. The GP PVDF Peptide method includes: ♦ A begin cycle to prepare the sample for sequencing ♦ A blank cycle ♦ A PTH-amino acid standard cycle ♦ Sequencing cycles The GP PVDF Peptide method delivers TFA vapor to the cartridge for cleavage of the ATZ-amino acid. A few microliters of BiobrenePlus solution diluted 1:10 with methanol should be applied to the sample membrane before sequencing.

Table 3-9 Standard Methods *(continued)*

Method	Used to...
Run Gradient	evaluate the performance and integrity of the HPLC system and the UV detector. The Run Gradient method activates the HPLC system and starts data collection without activating the Rheodyne injector valve.
PTH-Standards	verify the performance and integrity of the: ♦ HPLC system ♦ UV detector ♦ HPLC column ♦ Rheodyne injector valve and conversion flask ♦ Conversion reagents The PTH-standards method performs a complete conversion cycle on the PTH-standard mixture in the conversion flask, activates the HPLC system and injects the standard onto the HPLC column for separation and analysis.
Inject Optimize	adjust the volume of the sample in the flask after reconstitution and prior to loading into the HPLC sample loop.
Manual Injection	verify the performance and integrity of the: ♦ HPLC system ♦ UV detector ♦ HPLC column ♦ Rheodyne injector valve excluding the conversion flask ♦ Conversion reagents
Flask Optimize	optimize the flask drying steps, pre-conversion dry and post-conversion dry. The pre-conversion dry step concentrates the sample and evaporates the extraction solvents after transfer from the cartridge. The flask should not be completely dry after the pre-conversion dry step or loss of certain amino acid derivatives will result. The post-conversion dry step eliminates the 25% TFA used to convert the ATZ-amino acids to PTH-amino acids. The flask must be completely dry after the post-conversion dry step to avoid chromatographic disturbance.

Creating Functions, Cycles, and Methods 4

Overview

About This Chapter This chapter describes how to modify or create cycles and methods. A complete listing of the functions, cycles, and methods can be found in Appendix A and B.

In This Chapter This chapter contains the following topics:

Creating User-Defined Functions

Creating Special Functions

Functions 401–450 are allocated for user-defined functions.

IMPORTANT Functions cannot be created or modified when the sequencer is in use.

To create special functions:

Step	Action
1	Select the Functions view from the View pop-up menu.
2	Select one of the user functions using the up/down scroll button, and highlight the function.
3	Highlight the Function Name field, and type in the function name.
4	Move the cursor to the Valves Activated field.
5	Enter the valve numbers to be activated for the function. Enter a space between each valve number. **IMPORTANT** The number of valves that can be activated per function is limited by the following rules: Rule 1: For valves 1–23, 34–40, 45, 46, and 63, a maximum of 8 valves can be activated per function. For valves 24–33, 41–44, and 47–62, a maximum of 6 valves can be activated per function. Rule 2: A maximum of 14 valves total (8 cartridge and 6 flask) can be activated simultaneously.
6	Repeat steps 2 through 5 for all functions you create.
7	Pull down the File menu from the upper menu bar. Select Save Function to save all new functions.

Global Times

About Global Time

Most functions of the Procise® Protein Sequencer can be run with a global time setting. The global time is set from the Function view. For any cycle in which the Global time box is checked for a particular function, the Global time value from the Function view will be used as the time for that function.

In this way, the duration of a function can be modified wherever it is used in multiple cycles by making a single change in the Global time field. Standard cycles use global time only for the cartridge function Load X1 and the flask functions Pre-Conversion Dry, Post-Conversion Dry, and Concentrate Sample.

Setting Global Time

IMPORTANT Global times cannot be set when the sequencer is in use.

To set the global time:

Step	Action
1	Select the Function view from the View pop-up menu.
2	Highlight the function from the function list, and enter the desired global time in the global value field.
3	Pull down the File menu from the upper menu bar, and select Save Functions.

Activating Global Time

To activate the global time:

Step	Action
1	Use the View menu to select the Cycles & Procedures view.
2	Use the Cycle menu to select the type of cycle or procedure in which the global time will be activated. Then select the particular cycle to be modified.
3	Highlight the step where the global time will be activated.
4	Check the global box to activate the global time. An “X” in the global box indicates that the global time is active.
5	Pull down the File menu from the upper menu bar, and select Save Cycle/Procedure.

Creating Cycles

Modifying Existing Cartridge Cycles

To make a copy of the Procise standard cartridge cycle:

Step	Action
1	Use the View menu to select the Cycles and Procedures View.
2	Use the upper screen menu to select Cartridge Cycle for cycle type.
3	Use the lower screen menu to select the specific cycle to be copied.
4	Pull down the File menu from the upper menu bar, and select Save Cycle/Procedures As...
5	Type the new cycle name, and click the OK button.

Editing the Copied Cycle

To edit the copied cycle:

Step	Action
1	To delete a row, highlight the row to be deleted and click the Delete Row button.
2	To insert a row, select the function to be inserted from the function list on the left side of the screen. The function can be selected by using the scroll/down button or by typing the function number at the top corner of the function list. a. Highlight the row immediately before the intended insertion point. b. To enter the function run time, click on the global box to turn the global time off. Type the function time (in seconds) in the value box.

Saving the Edited Cycle

To save the edited cycle:

Step	Action
1	Pull down the File menu from the upper menu bar.
2	Select Save Cycle/Procedures.

IMPORTANT The Ready to Transfer step in a Cartridge cycle synchronizes with the Ready to Receive step in a Flask Cycle. The cartridge cycle must have Ready to Transfer and Transfer Complete steps to transfer sample to the flask.

The maximum number of steps allowed per cycle is 100.

Every cycle needs a Begin step and an End step.

Copying Existing Flask Cycles

To copy the Procise standard cartridge cycle:

Step	Action
1	Use the View menu to select the Cycles and Procedures View.
2	Use the upper screen menu to select Flask Cycle for cycle type.
3	Use the lower screen menu to select the specific cycle to be copied.
4	Use the File menu from the upper menu bar to select Save Cycle/Procedures As...
5	Type the new cycle name, and click the OK button.

Editing the Copied Flask Cycle

To edit the copied cycle:

Step	Action
1	To delete a row, highlight the row to be deleted and click the Delete Row button.
2	To insert a row, select the function to be inserted from the function list on the left side of the screen. The function can be selected by either using the scroll/down button or typing the function number at the top corner of the function list. a. Highlight the row immediately before the intended insertion point. b. To enter the function run time, click on the global box to turn the global time off. Type the function time (in seconds) in the value box.

Saving an Edited Flask Cycle

To save the edited cycle:

Step	Action
1	Pull down the File menu from the upper menu bar.
2	Select Save Cycle/Procedures.

IMPORTANT The Ready to Receive step in a Flask cycle synchronizes with the Ready to Transfer Step in a Cartridge Cycle. The flask cycle must have a Ready to Receive step to receive sample from the cartridge.

The Prepare Pump step initiates the pump to start running and equilibrates the column at the initial condition of the gradient to be run. Allow at least 8 minutes between the Prepare Pump and Load Injector steps.

The maximum number of steps allowed per cycle is 100.

Every cycle needs a Begin step and an End step.

Creating New Cycles Using a User Defined Cycle

To select User Defined Cycle 1:

Step	Action
1	Use the View menu to select Cycles and Procedures View.
2	Use the upper screen menu to select the cycle type.
3	Use the lower screen menu to select the User Defined Cycle 1.

Editing the New Cycle

To edit the new cycle:

Step	Action
1	To delete a row, highlight the row to be deleted and click the Delete Row button.
2	To insert a row, select the function to be inserted from the function list on the left side of the screen. The function can be selected by either using the scroll/down button or typing the function number at the top corner of the function list. a. Highlight the row immediately before the intended insertion point. b. To enter the function run time, click on the global box to turn the global time off. Type the function time (in seconds) in the value box.

Saving the New Cycle

To save the new cycle:

Step	Action
1	Pull down the File menu from the upper menu bar.
2	Select Save Cycle/Procedures As...
3	Type the new cycle name and click the OK button.

IMPORTANT The maximum number of steps allowed per cycle is 100.

A cartridge must have Ready to Transfer and Transfer Complete steps to receive sample from the cartridge.

Every cycle needs a Begin step and an End step.

Creating Methods

Copying a Standard Methods

To copy the standard method so that it can be edited:

Step	Action
1	Use the View menu to select Sequence Methods View.
2	Use the method screen menu to select the method to be copied.
3	Use the File menu from the upper menu bar, and select Save Method As...
4	Type the new method name, and click the OK button.

Editing the Default Method

To edit the default method:

Step	Action
1	Highlight the default method row. Select the new cartridge cycle, flask cycle, and/or gradient from each pop-up menu.
2	To delete a row, highlight the row to be deleted and click the Delete button.
3	To add a row, highlight the row after which the new row will be inserted and click the Insert Row button. a. Move the cursor to the Cycle # field and enter the cycle number to be added as an exception. b. Select the new cartridge cycle, flask cycle, and/or gradient from each pop-up menu.
4	If the cartridge, flask, or column temperatures need to be changed, move the cursor to the appropriate temperature field and enter the desired temperature.

Saving an Edited Method

To save the edited method:

Step	Action
1	Pull down the File menu from the upper menu bar.
2	Select Save Method.

IMPORTANT Nine exception cycles are allowed per method.

A cartridge cycle containing a Transfer to Flask step must be matched with a flask cycle containing a Ready to Receive step.

Creating New Methods

To create a new method:

Step	Action
1	Use the View menu to select Sequence Methods View.
2	Use the method screen menu to select the User Defined method.
3	Create the new method: a. Highlight the default method row. Select the new cartridge cycle, flask cycle, and gradient from each pop-up menu. b. To delete a row, highlight the row to be deleted and click the Delete button. c. To add a row, highlight the row after which the new row will be inserted and click the Insert Row button. – Move the cursor to the cycle # field and enter the cycle number to be added as an exception. – Select the proper cartridge cycle, flask cycle, and gradient for the cycle from each pop-up menu.

Saving the New Method

To save the new method:

Step	Action
1	Pull down the File menu from the upper menu bar.
2	Select Save Methods As...
3	Type the new name, and click the OK button.

IMPORTANT A method must contain a valid default cycle, which is the cycle that is run when there is no exception cycle. The default cycle is not necessarily a canned cycle.

A cartridge cycle containing a Ready to Transfer step must be matched with a flask cycle containing a Ready to Receive step.

Tests and Procedures 5

Overview

About This Chapter

In addition to sequencing cycles and methods, the Procise® system provides a number of test, cleanup, and diagnostic procedures. An explanation of the setup and execution of each test, as well as the method for creating new tests and procedures is in this chapter. A complete listing of the tests and procedures can be found in Appendix C.

In This Chapter

This chapter contains the following topics:

Running Tests

Using Test Procedures

All available tests and procedures can be run from the Tests view except bottle change procedures. Run bottle change procedures from the Bottle Change view.

IMPORTANT Tests and Procedures can not be run while the sequencer is active.

To use a test procedure:

Step	Action
1	Select the Tests View from the View pop-up menu.
2	Select a type of test by clicking the appropriate radio button.
3	<table><tr><th>If the...</th><th>Then...</th></tr><tr><td>Don't pause at error box is checked</td><td>the designated test(s) will be completed regardless of failure.</td></tr><tr><td>box is unchecked</td><td>a dialogue box will appear on the screen to bring attention to each failure as it arises. In this mode, it is necessary to manually resume the test(s) after each reported failure.</td></tr></table> In both cases, all test results are reported in the Event Log.
4	Use the Test pop-up menu and highlight the test procedure to be used. Multiple procedures can be selected by holding down the shift key.
5	Click on the Start Test button to run the selected test. The Stop Test button and the status field become active indicating that the test or procedure is running.
6	Press the Stop Test button to interrupt the procedure.

IMPORTANT Whenever possible, allow all Tests and Procedures to run to completion. Certain procedures adjust the pressure settings for reagent, solvent, or gas delivery. If the test or procedure must be interrupted, go to the Pressures and Temperatures view and make sure that all pressures are set to a value other than the operating value. If pressures have been set to zero, click on the Default button to restore original pressures.

Flow Test Procedures

Test Availability

There are seven flow test procedures for the Procise system. The procedure listed in Table 5-1 can be performed by users; the remaining procedures are used only during instrument manufacture and are not useful with the standard sequencing reagents installed.

Table 5-1 Flow Test Procedure

Flow Test	Usage
Sensor & Delivery Test	Check sensor activities

Sensor and Delivery Test Procedure

This procedure tests the operation of 11 fluid optical sensors. It delivers the chemical from the bottle through specific sensors and checks whether or not fluid was sensed within a function time. If fluid was not sensed, either the sensor is faulty or the delivery was incomplete. Any failures are reported in the event log.

Prior to starting this test, it might be necessary to run the Init Sensor procedure to set up the sensors for operation, if it has not been run automatically as a part of the method.

Note The test should be run while the designated sequencing chemicals are loaded on the instrument. In addition:

- X1 must contain 50:50 methanol:water.
- X2 must contain methanol.
- X3 must contain methanol.

See “Chemical Warnings Table” on page 1-7 for warnings about methanol solutions.

Once the procedure is complete, select the Event Log view from the View pop-up menu to determine whether any delivery errors have occurred.

Startup Procedures

About the Startup Procedure

A startup procedure is permanently stored in the Procise software. The startup procedure flushes each reagent/solvent bottle with argon and refreshes the reagent in the delivery line. The flask is washed with S4. No solvent or reagent is delivered through the cartridges. See “Chemical Warnings Table” on page 1-7 for warnings about argon gas and S4 solutions.

Startup procedures can be programmed from the Start Run view for the automatic run. The startup procedure is executed immediately after sensor initialization.

Programming from Start Run View

Program the startup and shutdown procedures from the Start Run view.

To program from the Start Run view:

Step	Action
1	Select the Start Run view from the View pop-up menu. At the bottom of the screen are pop-up menus for Startup and Shutdown procedures.
2	Click on the Startup pop-up menu at the left bottom of the window, and highlight the procedure to be used.
3	Select the Shutdown pop-up menu at the right bottom of the window, and highlight the procedure to be used.

Running A Startup Procedure

To run a startup procedure from the Test view:

Step	Action
1	Select the Test view from the View pop-up menu.
2	Click on the Startup button and select the Startup procedure.
3	Click on the Start Test button to run the procedure. **Note** Allow the procedure to run to completion.

Idle Procedures

About the Idle Procedure

When the instrument is not in use, oxygen diffuses slowly into the system and causes solvents and reagents to decompose and form by-products. The idle procedure is used to minimize sequencing problems due to chemical decomposition during an inactive period. The idle procedure flushes argon gas to each reagent/solvent bottle at a user-selectable level. See “Chemical Warnings Table” on page 1-7 for warnings about argon gas.

To execute the idle procedure:

Step	Action
1	Select Preferences from the Sequencer pull-down menu on the upper menu bar.
2	Click on the Execute Idle Procedure check box and enter the frequency at which the procedure should be run, ranging from once every hour to once every 999 hours.
3	**Note** It is not possible to pause or cancel an Idle Procedure once it is running. The sequencer cannot be programmed until the Idle Procedure run is complete. Select the Idle Procedure to be used from the menu and click on the OK button.
4	If the sequencer is active when the Idle procedure is selected, go to the Start Run view and click on the Update button.

Init Sensor Procedures

About the Init Sensor Procedure

The Procise system uses 11 optical sensors to detect fluid deliveries. Every time the Start Run button is clicked, the Procise system automatically runs the Init sensor procedure. This procedure flushes the flow path through each sensor and then takes a “dry” reading for each sensor. If the sensor light path is not completely dry, the sensor will not function correctly during sequencing.

IMPORTANT Always allow the Init Sensor procedure to run to completion. If the Procise sequencer has been shut down or if a sensor has been moved or replaced, the Init Sensor procedure must be run before sequencing or using manual control functions.

Whenever a sensor fails to sample sufficient consecutive wet readings before the end of a function, the information will be reported in the Event Log.

Leak Procedures

About Leak Tests

There are 18 leak tests or procedures in the Procise system software. Bottle and cartridge leak tests can be used routinely.

IMPORTANT Leak tests alter the pressure settings for reagent, solvent, and/or gas deliveries. If a test procedure is interrupted, pressure(s) may remain altered. Select the Pressures and Temperature view and click on the Default button to restore the original operating pressures.

Bottle Leak Tests

Bottle leak tests can be run directly from the Test view. Bottle leak testing is also available from the Bottle Change view by selecting the Bottle Change procedure followed by "-leak." Each bottle leak test reports the following three pressures:

- Pressurization: Check that the bottle can be adequately pressurized.
- Monitor Leak Rate: Measure the pressure drop with the regulator set to zero.
- Vent: Check the venting capability.

The results of each section of the leak test will be reported in Event Log at the end of the test. The actual bottle pressure must be within 0.05 psi of the target pressure in order to pass the leak test.

Cartridge Leak Test

The cartridge leak test ensures the leak tightness of each cartridge. It pressurizes the cartridge and monitors the leak rate. The test result will be reported in the Event Log at the end of the test. The actual cartridge pressure must be within 0.1 psi of the target pressure in order to pass the cartridge leak test.

Flask Leak Test

The flask leak test checks for leak tightness and venting capability of the flask assembly. The test result will be reported in the Event Log at the end of the test. The actual flask pressure must be within 0.05 psi of the target pressure in order to pass the flask leak test.

Valve Block Leak Test

The valve block leak tests check the leak tightness and venting capability of each valve block. The actual pressure held in the valve block must be within 0.05 psi of the target pressure in order to pass the valve block leak test.

Shutdown Procedures

Shutdown Procedures

Shutdown procedures can be programmed from the Start Run view for the automatic run. The shutdown procedure is executed after completion of the last cartridge scheduled to be run.

Two shutdown procedures are permanently stored in the Procise sequencer software:

- Post-Run Valve Block Wash – X3
- Short Term Shutdown Procedure

Post-Run Valve Block Wash – X3

This procedure is used to wash the system flowpaths from the X3 bottle position. Methanol should be loaded in the X3 position before starting this procedure. See “Chemical Warnings Table” on page 1-7 for warnings about methanol.

This procedure does not deliver solvent through the reaction cartridges or to other bottles.

Short Term Shutdown Procedure

This procedure is used when the Procise system will be idle for one to two weeks. During the short term shutdown procedure, ethyl acetate (S2B) is used to wash all valve blocks, delivery lines and loops. The flask and injector are washed with S4. After common flow paths are washed and flushed with argon, each bottle is briefly backflushed with argon to move reagents out of the Teflon delivery line. See “Chemical Warnings Table” on page 1-7 for warnings about S2B, S4, and argon.

To run a Shutdown procedure from Test view:

Step	Action
1	Select the Test view from the View pop-up menu.
2	Click on the Shutdown button and select the Shutdown procedure.
3	Click on the Shutdown button to run the procedure.
4	Allow the procedure to run to completion.

Cleanup Procedures

Three Cleanup Procedures

Three cleanup procedures are programmed in the Procise control software:

- Delivery Line Back-flush
- System Clean-out – X3
- System Flush – Argon

Delivery Line Back-flush

When the Procise system is to be cleaned, first use the delivery line back-flush procedure to prepare the system for removal of all reagent bottles. This procedure back-flushes all reagents and solvents to clean up all delivery lines. When this procedure is finished, take off all reagent/solvent bottles and empty them. Then, put empty bottles on the Procise system before running the System Clean-out – X3 procedure.

System Clean-out – X3

This procedure cleans the entire Procise system. Place a bottle of 100% methanol bottle at the X3 bottle position. All valve blocks, delivery lines, reaction cartridges, loops, injectors, and reagent bottles are washed with methanol. See “Chemical Warnings Table” on page 1-7 for warnings about methanol.

IMPORTANT The System Clean-out – X3 procedure alters the pressure management system pressure settings. When this procedure is finished, select the Pressure & Temperature view and click the Default button to restore the operating pressures.

System Flush – Argon

This procedure will completely flush and dry all Procise sequencer flow paths with argon. See “Chemical Warnings Table” on page 1-7 for warnings about argon.

Regulators are reset to default pressure settings during this procedure.

Electrical Test Procedure

This procedure tests the electrical continuity of key components in the Procise system. At step #7, the Rheodyne switches position from load to injection.

Bottle Change Procedures

The Process

Bottle Change Process

Stage	Description
1	Starts with back-flushing a specific chemical into the reagent bottle.
2	The reagent bottle is vented.
3	The new bottle is loaded.
4	The bottle is flushed with argon gas.
5	The chemical is delivered to the waste bottle, and washes the associated valve blocks and Teflon lines.

Two Procedures

Two procedures are available for each bottle position:

- A bottle change only
- A leak test on the bottle position as well as a bottle change

Starting the Bottle Change Procedure

Note The bottle change procedure can only be used when the Procise system is idle or paused.

To start the bottle change procedure:

Step	Action
1	Select the Bottle Change view from the View pop-up menu.
2	Highlight the desired procedure from the bottle change procedure pop-up menu.
3	Click the OK button.

Table of Bottle Changes

Table 5-2 Bottle Change and Cycle Time

Procedure	Cycle Time (min.)
Bottle Change for R1	1:35
Bottle Change for R1 - leak	2:45
Bottle Change for R2	1:00
Bottle Change for R2 - leak	2:00
Bottle Change for R3	3:35
Bottle Change for R3 - leak	4:50
Bottle Change for R4	1:35
Bottle Change for R4 - leak	3:00
Bottle Change for R5	1:25
Bottle Change for R5 - leak	2:50
Bottle Change for S1	1:30
Bottle Change for S1 - leak	2:55
Bottle Change for S2	1:15
Bottle Change for S2 - leak	2:40
Bottle Change for S3	1:15
Bottle Change for S3 - leak	2:40

Table 5-2 Bottle Change and Cycle Time *(continued)*

Procedure	Cycle Time (min.)
Bottle Change for S4	1:15
Bottle Change for S4 - leak	2:40
Bottle Change for X1	3:25
Bottle Change for X1 - leak	4:50
Bottle Change for X2	2:05
Bottle Change for X2 - leak	3:30
Bottle Change for X3 both	5:45
Bottle Change for X3 both - leak	7:10

Changing a Bottle

IMPORTANT Leak tests use functions that alter the pressure management system pressure setting. If the procedure is aborted before the end step, select the Pressure & Temperature view and click on the Default button to restore the operating pressures.

To change a bottle:

Step	Action
1	Select the Bottle Change View from the View pop-up menu. Do not remove the bottle at this time.
2	Highlight the chemical to be changed.
3	Enter the lot number of the chemical in the Lot Number box. (The date will be updated automatically.)
4	Click on the Change Bottle button.
5	a. When prompted, remove the old bottle and bottle seal. b. Put a new bottle seal on the new bottle rim. c. Screw the bottle into the bottle cap assembly.
6	Tighten until the bottle seal contacts the top of the bottle cap assembly and then turn approximately a quarter-turn more. **IMPORTANT** It is not necessary to tighten a bottle until a snapping sound (ratcheting) is produced by the bottle cap assembly. Ratcheting the bottle cap assembly will cause premature wear and may crack the bottle seal.
7	Click on the Continue button. The bottle change procedure will continue through the remaining steps, which includes priming the delivery line up to the valve block.
8	If necessary, load the additional chemicals from the list in the same manner.
9	Pull down the File menu from the upper menu bar and select Save Chemicals when the bottle changes are completed.

Creating Tests and Procedures

Copying a Test or Procedure

Copy the standard tests and procedures so that they can be edited.

To make a copy:

Step	Action
1	Use the View pop-up menu to select Cycles and Procedures View.
2	Use the upper screen menu to select the type of procedure to be edited.
3	Use the lower screen menu to select the specific procedure to be copied.
4	Pull down the File menu from the upper menu bar and select Save Cycle/Procedures As…
5	Type the new procedure name and click on the OK button.

Editing the Copy

To edit the copied procedure:

Step	Action
1	To delete a row, highlight the row to be deleted and click on the Delete Row button.
2	To insert a row, select the function to be inserted from the function list on the left side of the window. (The function can be selected by either using the scroll/down button or typing the function number at the top corner of the function list.)
3	Highlight the row immediately before the intended insertion point.
4	Check off the global box if the global time not is used. Type the function time (in seconds) in the value box.

Save the Edited Procedure

To save the edited procedure:

Step	Action
1	Pull down the File menu from the upper menu bar.
2	Select Save Cycle/Procedure.

IMPORTANT The maximum number of steps allowed per cycle is 100.

Every cycle needs a Begin step and End step at the beginning and end of the cycle, respectively.

Chemical Optimization 6

Overview

About This Chapter

This chapter provides guidelines for the development and optimization of functions, cycles, and methods for the Procise® Protein Sequencer. It includes explanations of the optimization of loop loadings, deliveries, and washing and drying steps for both the cartridge and the flask.

In This Chapter

This chapter contains the following topics:

Optimizing the Injector

Reconstituting the Sample

During flask cycles, a sample is reconstituted in 10% acetonitrile (S4C). This ensures that hydrophobic amino acids will go into solution. For consistent chromatography, however, the percentage of acetonitrile must be reduced before sample injection.

Reducing Acetonitrile Content

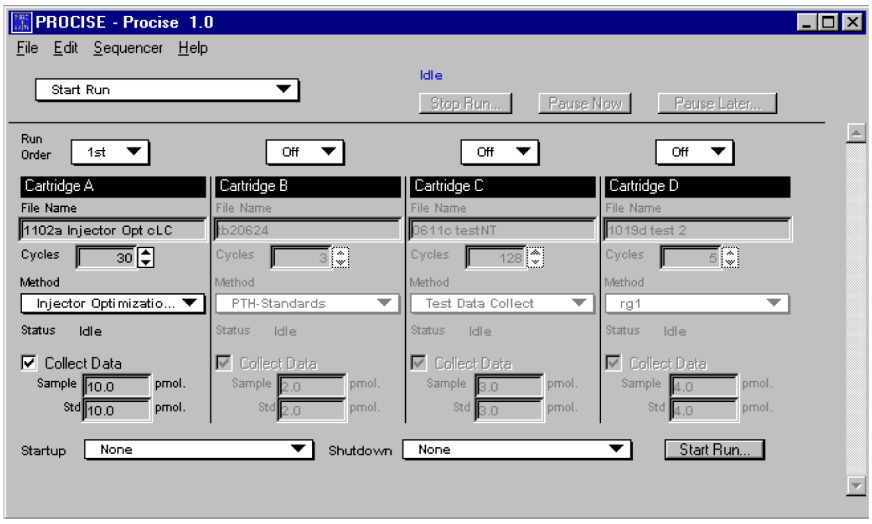
To reduce acetonitrile content, two Concentrate Sample steps are included in all flask cycles. The duration of these steps determines the percentage of sample injected. Therefore, the duration of the Concentrate Sample steps must be optimized so that the correct amount of sample is injected.

Optimize the injector percentage if a large number of Sample Loop Full error messages are recorded in the event log.

Setting Up the Protein Sequencer

Two procedures are provided here, one to set up the protein sequencer for optimization and the second to perform optimization.

To set up the protein sequencer for optimization:

Step	Action
1	Scroll to the Functions dialog box.
2	Ensure that the global time for the Concentrate Sample step (step 238) is 100 sec.
3	Place a mark 1 in. from the hexagonal tip of the 5/16-in. bushing on the yellow tubing connected to valve block port 42.
4	Scroll to the Start Run dialog box. 
5	Set up a run with the following conditions: **Parameter** / **Setting** Cartridge A: 1st Filename: Your choice Number of cycles: 10 Method: Injector Optimization

To run the optimization procedure:

Step	Action
1	Click Start Run. The Init Sensor procedure will start running. You can click Jump to advance to the last step of this procedure.
2	If the flask temperature is 64 °C when the run pauses at the Begin step of the flask cycle, click Next Step to start the Injector Optimization method.
3	Click Pause Later, and configure the run to pause on Cartridge A at the end of the first cycle.
4	At the end of the first cycle, look for the injection slug in the plumbing line. Ideally, the *end* of the slug should be between the mark you made on the tubing and the valve block. It should not be in the pickup line connected to port 41.
5	If the end of the injection slug is not in the correct location, modify the Concentrate Sample time global value in the Functions dialog box. Increase or decrease the value as appropriate in 5-sec increments only. **IMPORTANT** Do not increase the Concentrate Sample step time by more than 5 sec at a time. Otherwise, an air injection might occur. An air injection will damage the column.
6	Click Resume.
7	Once you have determined the correct value for the Concentrate Sample step, run at least one more cycle to confirm the optimization.

Optimizing Flask Dry Times

Overview

Set up a protein sequencer run to optimize the global time values for the Pre- and Post-Conversion Dry steps in all flask cycles.

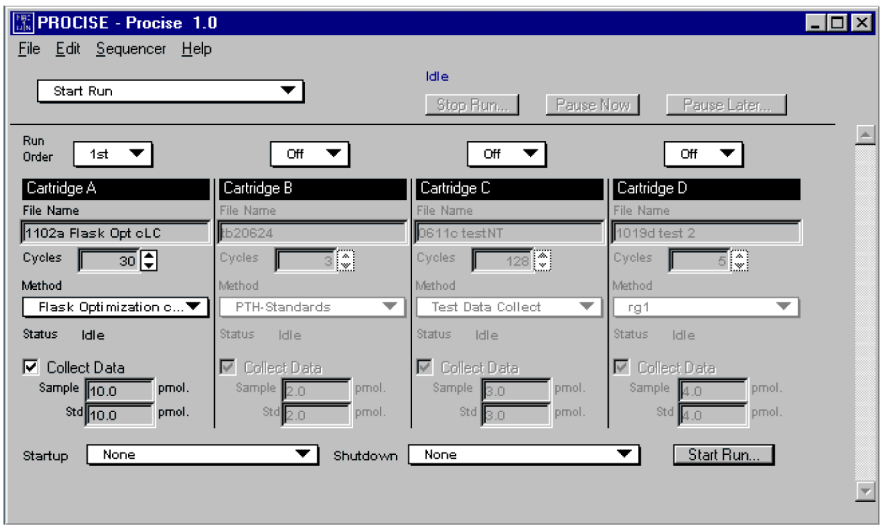
Note We recommend that you optimize these values whenever the flask and pickup line are cleaned or replaced.

Optimizing The Global Time Values

The following procedures are provided here:

- A procedure for setting up the protein sequencer
- A procedure for starting a run
- A procedure for continuing the run
- A procedure for calculating the optimized pre- and post-conversion dry step times
- A procedure for verifying the optimized settings

To set up the protein sequencer:

Step	Action
1	Install a reaction cartridge in the cartridge A position on the protein sequencer.
2	Perform a leak test on cartridge A.
3	Scroll to the Start Run dialog box. 
4	Configure cartridge A as follows: **Parameter** / **Setting** Cartridge A: 1st Filename: Your choice Number of cycles: 5 Method: Flask Optimization

To start the run:

Step	Action
1	Click Start Run. The Init Sensor procedure begins. You can click Jump to advance to the last step of this procedure.
2	Once the cartridge and flask have reached the proper temperatures, the Flask Optimization method begins.
3	At the first pause, check the flask for liquid. The flask should not be completely dry. If the flask is not dry, proceed to step 4. **If...** / **Then...** the flask is dry / check the pressure settings on the protein sequencer, which should be set to the default values. the pressure settings are not correct / reset them to the default settings, and start the procedure over.
4	Click Resume.
5	Click Hold as soon as the Pre-Conversion step begins. When the flask contents visibly stop bubbling (when there are between 5 and 10 µL of liquid remaining in the flask), note both the Time and Remaining values shown. Be sure to mark these as Pre-Conversion Dry values.

To continue the run:

Step	Action
1	Click Next Step twice.
2	At the next pause, click Resume.
3	Click Hold as soon as the Dry Flask step begins.
4	When the flask is visibly dry, note both the Time and Remaining values. Be sure to mark these as Post-Conversion Dry values.

To calculate the optimized pre- and post-conversion dry step times:

Step	Action
1	Calculate the optimum time for the Pre-Conversion Dry step by subtracting the Remaining value from the Time value. Pre-Conversion Dry time = Time value – Remaining value + 105
2	Calculate the optimum time for the Post Conversion Dry step as follows: Post-Conversion Dry time = [Time value – Remaining value] – 250 The result must be a positive number. If it is not, ensure that the flask is set to the correct temperature and that two full loads of S4 are being delivered to the flask during the procedure.
3	Scroll to the Functions dialog box.
4	Change the global value of Function 236, Pre-Conversion Dry, to the optimized value you calculated.
5	Change the global value of Function 237, Post-Conversion Dry, to the optimized value you calculated.

Verifying The Optimized Settings

To set up the protein sequencer for a run:

Step	Action
1	Install a reaction cartridge in the cartridge A position on the protein sequencer.
2	Perform a leak test on cartridge A.
3	Scroll to the Start Run dialog box, and configure cartridge A as follows:

Parameter	Setting
Cartridge A	1st
Filename	Your choice
Number of cycles	1
Method	Flask Optimization

To verify the optimization:

Step	Action
1	Click Start Run. The Init Sensor procedure begins. You can click Jump to advance to the last step of this procedure.
2	Once the cartridge and flask have reached the proper temperatures, the Flask Optimization method begins.
3	At the first pause, Click Resume.
4	At the next pause, check the flask for liquid. The flask should still contain 5 –10 µL of liquid (approximately 1/3 of the conical section of the flask).
5	At the next pause, click Resume, and continue watching the liquid dry in the flask.
6	Once the flask is visibly dry, note the amount of time that elapses from this point to the end of the step. The elapsed time should be approximately 200 sec.
7	Click Stop Run to end the cycle.

IMPORTANT If the verification was not successful, perform the optimization procedure again.

Optimizing Sensor Functions

About the Sensors

Sensor functions are specialized valve-controlling functions. These functions are controlled by the target sensor, rather than by a specific time setting. When a function is activated, the sensor begins “looking” for fluid. When fluid is detected by the sensor, the reagent or solvent delivery valve is turned off. The time for the function continues to count down to zero and the next step begins.

In order for a sensor function to perform correctly, enough time must be allotted in the step for fluid to reach the sensor. If fluid reaches the sensor within the allotted step time, there is no report to the event log. If fluid does not reach the sensor within the allotted time, an error will be reported in the Event Log and the protein sequencer may be paused.

Each optical fluid sensor used in the protein sequencer consists of an infrared emitting diode and photo-sensor receiver. Fluid is detected by increased light transmission through the Teflon tube due to the change in refractive index.

Sensor Types

The Procise Protein Sequencer uses 11 sensors:

- Cartridge Load 1 (Small) Sensor
- Cartridge Load 2 (Large) Sensor
- Cartridge A Outlet Sensor
- Cartridge B Outlet Sensor
- Cartridge C Outlet Sensor
- Cartridge D Outlet Sensor
- Flask Load 1 (Small) Sensor
- Flask Load 2 (Large) Sensor
- Transfer to Flask Sensor
- Sample Loop Load Sensor
- Sample Loop Full Sensor

Sensors and the Event Log

Error Message

If a sensor does not detect fluid during the time allotted for the function, an error message will be posted in the Event Log. For 9 of the 11 sensors, the Procise software will display a dialog box describing the failure and the protein sequencer will pause at the end of the active cycle unless the operator is present to intervene. The Transfer to Flask Sensor and the Sample Loop Full Sensor are the exceptions. Failure of these sensors to detect fluid results only in the posting of an error in the Event Log.

Example of Event Log Entry

An example of the information reported to the Event Log:

01/01/1995 4:30:00 PM

During step 2 of cycle 1, fluid was not detected by the

Cartridge Load 2 (large)

Sensor

The protein sequencer will pause at end of this cycle.

(Dry = 500, Threshold = 750, Average wet = xx)

dry	wet	dry	wet	dry	wet
(xx,	xx,	xx,	xx,	xx,	xx,
xx,	xx,	xx,	xx,	xx,	xx,
xx,	xx,	xx,	xx,	xx,	xx,)

Interpreting the Event Log Information

The information provided by the Event Log is as follows:

- The date and time of the failure
- The step number and cycle number of the failure and the name of the sensor reporting the failure
- Whether or not the protein sequencer will be paused

The next entries are:

(Dry = 500, Threshold = 750, Average wet = xx)

Item	Description
Dry = 500	The empty tube transmission (dry) reading from the sensor (generated during the Init Sensor procedure)
Threshold = 750	The minimum transmission value necessary for a sensor reading to be considered “wet” (dry reading multiplied by 1.5)
Average wet = xx	Actual transmission reading with fluid in tube (if no fluid is detected, average wet = 0)

The last entry table is:

dry	wet	dry	wet	dry	wet
(xx,	xx,	xx,	xx,	xx,	xx,
xx,	xx,	xx,	xx,	xx,	xx,
xx,	xx,	xx,	xx,	xx,	xx,)

These values represent the number of dry and wet readings taken by the sensor. Each sensor requires a certain number of wet readings to discriminate the arrival of the expected reagent or solvent from a stray droplet of fluid in the line. If fluid never reaches the sensor, only the first dry field will have a non-zero value.

Error Message Detecting No Flow

The following error message will be observed if a bottle runs dry during a run, or if there is a restriction to reagent or solvent flow in the delivery path.

dry	wet	dry	wet	dry	wet
(5000,	0,	0,	0,	0,	0,...)

Error Message Detecting Bubbles

If the dry/wet values reported in the event log are as follows, then the sensor is detecting very small bubbles in the solvent or reagent stream. This is a result of the solvent or reagent degassing as it flows through the valve blocks and can usually be corrected by reducing the bottle pressure for the particular chemical.

dry	wet	dry	wet	dry	wet
(5000,	58,	1,	47,	2,	53,...)

Optimizing Cartridge Load Functions

Two Cartridge Load Loops

Two load loops are available for metering reagents to the cartridge:

- A large loop which loads a nominal 10 µL of any cartridge reagent
- A small loop which loads a nominal 5 µL of any cartridge reagent

The standard instrument cycles use the large loop for loading the cartridge reagents.

Loop	Usage
Large loop	Delivers a volume of reagent that wets but does not saturate a glass fiber sample disk in the microcartridge (9 mm disk size). The large loop volume of reagent is appropriate for blot samples, whether run in the blot cartridge or a microcartridge.
Small loop	A small loop load of TFA may be preferable for small pieces of PVDF or for smaller volume reaction cartridges.

Determining Cartridge Load Function Time

Note Whenever the delivery pressure for a reagent is changed, load times must be changed as well.

Note If the protein sequencer has not been run since the last cold start, use the Test view to select and run the Init Sensor procedure. Then allow the procedure to run to completion.

To determine the amount of time necessary for a cartridge load function:

Step	Action
1	From the Pressures and Temperatures view, set the desired delivery pressure for the bottle position to be used.
2	If the reagent or solvent is not loaded on the instrument, perform the bottle change procedure for that bottle position.
3	From the Manual Control view, select Function 139, Flush Large Loop, or Function 140, Flush Small Loop, from the cartridge function list. Activate the appropriate flush function for 20 seconds to make sure that the loop is clear of any liquid.
4	a. Activate the load function for the bottle and loop of choice. (For example, select Function 183 to load the large loop with reagent or solvent from the X2 bottle position.) b. Watch for the appearance of a check mark next to the reaction flow sensor field at the top of the screen. c. Note the elapsed time and add 5–10 seconds. Enter this load time in the cycle for this function. d. Select “All Off” before leaving the Manual Control view.
5	From the Function view, enter the load time in the global time field for that function. Once the time for loading the large loop has been determined, the same value can be used for loading the small loop.

Rules to Apply to Cartridge Load Functions

The following rules apply to using cartridge load functions in a custom cycle:

- The loop must be flushed for at least 10 seconds before the first loading.
- The loop must be flushed for at least 5 seconds between loadings.
- The loop must be washed and flushed between loadings of multiple reagents.

Optimizing Flask Load Functions

Two Flask Load Loops

There are two load loops available for the flask. Unlike the cartridge load loops, the volume of any particular reagent or solvent loaded depends on the position of that chemical on the valve block.

Reagent/Solvent	Small Loop (µL)	Large Loop (µL)
S4	25	60
X3	20	55
X2	15	50
R4	10	45
R5	5	40

Determining Flask Load Function Time

IMPORTANT Whenever the delivery pressure for a reagent is changed, load times must be changed as well.

IMPORTANT If the protein sequencer has not been run since the last cold start, use the Test view to select and run the Init Sensor procedure. Then allow the procedure to run to completion.

To determine the amount of time necessary for a flask load function:

Step	Action
1	From the Pressures and Temperatures view, set the desired delivery pressure for the bottle position to be used.
2	If the reagent or solvent is not loaded on the instrument, perform the bottle change procedure for that bottle position.
3	a. From the Manual Control view, select Function 217, Flush Large Loop, or Function 218, Flush Small Loop, from the flask function list. b. Activate the appropriate flush function for 20 seconds to make sure that the loop is clear of any liquid.
4	a. Activate the load function for the bottle and loop of choice. (For example, select Function 75 to load the small loop with reagent or solvent from the X1 bottle position.) b. Watch for the appearance of a check mark next to the reaction flow sensor field at the top of the screen. c. Note the elapsed time and add 5–10 seconds. d. Enter this load time in the cycle for this function. e. Select “All Off” before leaving the Manual Control view.
5	From the Function view, enter the load time in the global time field for that function. Once the time for loading the large loop has been determined, the same value can be used for loading the small loop.

Rules to Apply to Flask Load Functions

The following rules apply to using flask load functions in a custom cycle:

- The loop must be flushed for at least 10 seconds before the first loading.
- The loop must be flushed for at least 10 seconds between loadings.
- The loop must be washed and flushed between loadings of multiple reagents.

Optimizing “Deliver to Cartridge” (Sensor) Functions

Cartridge Outlet Sensors

A liquid sensor at the outlet of each cartridge simplifies optimization of the delivery of solvent to the cartridges for both washing and extraction. The sensor eliminates the need for timing the delivery of solvent to the “midpoint” of the cartridge. All washes and extractions in the standard cycles, except for the wash after cleavage, are controlled by these sensors.

Washings are controlled by a first delivery to the cartridge outlet sensor followed by short pulses of solvent alternated with wait steps. Extractions are deliveries to the cartridge outlet sensor, followed by a brief incubation and transfer to the flask.

Determining Deliver to Cartridge (Sensor) Function Time

Note Whenever the delivery pressure for a reagent is changed, load times must be changed as well.

IMPORTANT If the protein sequencer has not been run since the last cold start, use the Test view to select and run the Init Sensor procedure. Then allow the procedure to run to completion.

To determine the amount of time necessary for a Deliver to Cartridge (sensor) function:

Step	Action
1	From the Pressures and Temperatures view, set the desired delivery pressure for the bottle position to be used.
2	If the reagent or solvent is not loaded on the instrument, perform the bottle change procedure for that bottle position.
3	From the Manual Control view,: a. Select Function 131, Dry Cart (top) from the cartridge function list. b. Activate the function for 40 seconds to make sure that the sensor light path is clear of any liquid.
4	a. Activate the Deliver to Cartridge (sensor) function for the bottle or solvent of choice. (For example, select Function 75 to deliver reagent or solvent from the X1 bottle position to the cartridge outlet sensor.) b. Watch for the appearance of a check mark next to the reaction flow sensor field at the top of the screen. c. Note the elapsed time and add 5–10 seconds. Enter this load time in the cycle for this function. d. Select “All Off” before leaving the Manual Control view.
5	From the Function view, enter the load time in the global time field for that function. Once the time for loading the large loop has been determined, the same value can be used for loading the small loop.

Rules to Apply to Using Cartridge Load Functions

The following rules apply to using cartridge load functions in a custom cycle:

- The cartridge must be flushed for at least 40 seconds before the first delivery.
- The cartridge must be flushed for at least 40 seconds between deliveries.

Chemistry Optimization

Overview

This section helps explain the various processes used during Edman chemistry and what is necessary for the creation or optimization of chemistry cycles. See “Chemical Warnings Table” on page 1-7 for warnings about reagents or solutions mentioned in the following paragraphs.

The goal of a protein sequencing run on an unknown sample is to unambiguously identify as many amino acids as possible using the least amount of sample. The length of a protein sequence that can be determined is limited by the chemical efficiency of the Edman degradation as well as the purity, amount and molecular weight of the sample to be sequenced. Because the chemical efficiency is less than 100%, the amount of sample you can sequence decreases slightly with each successive degradation cycle.

Cleavage Efficiency

With the exception of the initial coupling, the reaction of PITC with the amino-terminus or termini proceeds nearly quantitatively. The particular amino acid being reacted or the local structure of the peptide chain has little effect on the efficiency of the coupling reaction. The cleavage reaction requires strong acid, however, so a balance must be struck between complete cleavage of the ATZ-amino acid from the peptide and unwanted acid cleavage at other sites along the peptide chain. Because of this balance, cleavage efficiency is affected by the amino acid derivative being cleaved as well as the next amino acid in the chain.

Incomplete cleavage of the ATZ-amino acid is referred to as “lag” and the remaining, uncleaved portion of the current N-terminal amino acid will appear in the chromatogram for the next cycle along with the next amino acid. Lag increases with each cycle in a sequencing run, and depending on the particular amino acids in the sequence, may be the primary reason for a sample to stop producing useful sequence data.

Amino Acid Background

Repetitive exposure of the sample to strong acid during the cleavage reaction can cause cleavage between amino acids elsewhere in the peptide chain. Each time “non-specific” cleavage of the peptide chain occurs, a new N-terminus is generated which can react with PITC. This will cause an increase in the “amino acid background,” the presence of other PTH-amino acids in the chromatogram which do not reflect the true N-terminal sequence.

At the start of a sequencing run, the amino acid background from non-specific cleavage is low. This background increases with each sequencing cycle. Fortunately, non-specific cleavage is sequence specific, so only peptide bonds between amino acids will be cleaved. This keeps the amino acid background rate from cycle to cycle quite low. However, for proteins with labile amino acid sequences and very large proteins, amino acid background will increase much more rapidly. In practical terms, while 50 picomoles of a 100–200 amino acid protein may provide 40–50 cycles of interpretable sequence, the same amount of a 2000 amino acid protein will probably provide only 10–15 cycles of sequence.

Coupling

About the Coupling Process

Coupling is the process of reacting the free amino-terminus of a protein or peptide sample with PITC to create a phenylthiocarbamyl (PTC) protein/peptide. It includes:

- Delivery of PITC
- Delivery of base vapor to provide the basic environment necessary for coupling
- Drying and washing to remove excess reagent and reaction by-products

The coupling reaction used for samples bound to PVDF membrane is slightly different from the coupling for samples applied to glass fiber. Sequencing cycles typically have been written for samples applied to a hydrophilic support. The hydrophilicity of the support facilitates the absorption of a small amount of water necessary for efficient coupling of PITC to the amino-terminus of the sample.

PVDF membrane is routinely used for electroblotting of samples from gels and can be used for the clean-up of salt and buffer containing samples prior to loading on the protein sequencer. PVDF membrane binds proteins through hydrophobic interaction. Because PVDF membrane is hydrophobic, its tendency is to repel water rather than absorb it.

In the coupling reaction of the blot cycles, an aliquot of 50% methanol in water is delivered from the X1 bottle position to the reaction cartridge in order to wet the membrane prior to coupling. A brief argon drying step removes the methanol, leaving the sample solvated with a small amount of water when the coupling reaction begins. This addition improves the coupling efficiency for PVDF membrane-bound samples.

PITC Delivery

All the standard chemistry cycles use three deliveries of PITC during the coupling reaction. After each PITC delivery there is a short argon delivery to evaporate the heptane. Residual heptane would interfere with the reaction of PITC and the sample by keeping most of the PITC in the organic phase. The drying time should be at least 20 seconds to insure adequate removal of the heptane.

It is possible to use two PITC deliveries for coupling rather than three. If two PITC deliveries are used, the base deliveries should be increased to 270–300 seconds each. It is also possible to use more than three PITC deliveries if necessary. Examples include a very large amount of sample being sequenced, or multiple pieces of PVDF where contact of reagent and membrane may be of concern. A base delivery should always precede the first PITC delivery to the cartridge.

Coupling Base Delivery

As a first step of coupling in all chemistry cycles, R2B vapor is delivered to the cartridge to raise the pH of the sample and deprotonate the free amino-groups for reaction with PITC. The length of this delivery should be at least 20–30 seconds but can be increased to as much as 120 seconds without negative impact. The length of the base deliveries after the PITC delivery can be adjusted but should be at least 120 seconds. Avoid making the total base delivery time longer than 700 seconds to minimize the modification of aspartic and glutamic acid residues.

Under the basic conditions necessary for coupling, aspartic and glutamic acid residues are slowly modified by reaction of the side chain carboxylic acid group with aniline. The derivative of Asp can be found just before the DPTU peak in the chromatogram; the derivative of Glu is just after DPTU. The extent of modification of Asp and Glu residues increases slightly with each sequencing cycle. The effect is

more pronounced for Glu residues. The rate of modification of Asp and Glu residues also increases with the coupling temperature and is most noticeable for relatively large (>100 picomoles) amounts of sample.

Coupling Temperature

The temperature of the cartridge during coupling is set high enough to promote fast, efficient reaction of PITC with the amino-terminal amino group without excessive side-reactions. For example, the standard pulsed-liquid cycles use a coupling temperature of 45 °C, while the pulsed-liquid blot cycles use a 48 °C coupling temperature. Under the basic conditions necessary for coupling, Asp and Glu acid residues are slowly modified by reaction of the side chain carboxylic acid group with aniline.

The rate of modification of Asp and Glu residues is slightly higher on glass fiber than on PVDF. The lower coupling temperature for glass fiber provides a rate comparable to PVDF at the higher temperature. The rate of modification of Asp and Glu residues also increases with the length of coupling and is most noticeable for relatively large (>100 picomoles) amounts of sample.

Drying After Coupling

Drying after coupling primarily eliminates the water absorbed by the polybrene during the coupling reaction. Some of the reaction chemicals will also be reduced during this step, but the subsequent wash will remove the bulk of the chemistry by-products. This drying can be extended with no concern for the loss of recovery of any residues. The goal is to eliminate as much water as possible before the wash and cleavage to prevent sample washout and hydrolysis of the peptide chain during the cleavage.

Post-coupling Wash

The purpose of the post-coupling wash is to remove as much of the coupling reagents and reagent by-products as possible before the cleavage reaction begins. The washing is done with a combination of the solvents S2B and S3.

The washing scheme of short deliveries of solvent alternating with brief cartridge wait steps reduces the likelihood of sample washout and provides the best efficiency of washing with minimal solvent consumption.

The first delivery of solvent to the cartridge is S3, the less polar of the solvents, to reduce the possibility of sample being washed out of the reaction cartridge. Increasing the volume of solvent used for post-coupling washing will reduce the chemistry background, but may increase the loss of sample from the cartridge due to washout, particularly if short hydrophobic peptides are being sequenced. In particular, lengthy S2B washings will aggravate sample washout.

Drying after the post-coupling wash should completely dry the sample to prevent loss to washout but does not require any other special considerations. Typically there is no danger of over-drying the sample at this point.

Cleavage

About the Cleavage Process

Cleavage, whether pulsed-liquid or gas-phase, is the TFA-catalyzed process of removing the PTC-amino acid from the amino terminal end of the sample. Under strongly acidic conditions, the peptide chain is cleaved at the peptide bond nearest to the PTC-amino acid derivative, resulting in the release of an ATZ-amino acid. The cleavage is not a hydrolytic process, so ideally the sample should be as free of water as possible to minimize non-specific hydrolytic cleavage of the peptide chain.

Pulsed-liquid Cleavage

Pulsed-liquid cleavage is performed by delivering a small aliquot of TFA to the cartridge on a stream of argon and sealing off the reaction chamber by closing the valves into and out of the cartridges to allow the cleavage to take place. Pulsed-liquid cleavage proceeds faster than gas-phase cleavage. The standard pulsed-liquid cleavage time is 300 seconds at 48 °C.

Certain samples may benefit by varying the cleavage conditions. For example, very large protein samples may sequence better using a shorter cleavage time to minimize amino acid background generated from non-specific cleavage of certain peptide bonds. Cleavage of the peptide bond after certain amino acids, particularly proline, proceeds more slowly than others and will benefit from an extended cleavage time or increased cleavage temperature. Cleavage for proline residues can be extended up to 600 seconds, twice as long as a standard cleavage. Alternatively, the temperature of the cleavage can be increased to 55 °C. These extreme cleavage conditions should be used only where needed as the rate of sample degradation would be significantly increased if these conditions were used for every cycle.

Gas-phase Cleavage

Gas-phase cleavage is performed by delivering TFA vapor through the active cartridge for a prescribed period of time in order for the cleavage to take place. Gas-phase cleavage requires more time than pulsed-liquid phase cleavage and as a result, the standard gas-phase cycles are approximately five minutes longer than their pulsed-liquid counterparts. For the best results using the gas-phase cycles, it may be necessary to reduce the R3 pressure setting to 0.8–1.0 psi.

Too high a TFA flow rate through the cartridge will result in higher than expected lag. If the sequencing lag per cycle for a sample using the gas-phase cleavage cycle is higher than when using the pulsed-liquid cycle, then the R3 regulator pressure should be reduced. Gas-phase cleavage cycles tend to be somewhat cleaner than pulsed-liquid cycles, that is, the level of chemistry artifact peaks is usually slightly lower. Gas-phase cleavage may help reduce washout of hydrophobic peptides.

Drying After Cleavage

Drying after cleavage must balance between recovery of particular residues (especially the basic residues whose recoveries are drastically reduced if the sample is overdried) and excessive washout (if the sample is still too acidic when the extractions are done). After cleavage, care should be used to prevent overdrying of the sample which could result in poor extraction of charged residues and dehydration of labile residues.

In the standard pulsed-liquid cycles, 40 seconds of drying time is used. Incomplete drying may cause lowered repetitive yields due to sample washout. If sample washout is a greater concern than recovery of positively charged residues, the drying after cleavage can be extended.

ATZ Extraction and Transfer

After the cleavage of the ATZ-amino acid from the peptide chain is complete and the sample dried, the ATZ-amino acid is extracted from the cartridge and transferred to the flask so that the coupling of the new amino-terminus of the sample can begin. The best method for the ATZ extraction is slightly different for the various sample types.

Liquid Samples

Samples applied to glass fiber disks with polybrene, whether sequenced using gas or liquid cleavage, are extracted in the same way. Each glass fiber cycle has two ATZ extractions, the first extraction is done with S3 (butyl chloride) the second with S2B (ethyl acetate) and the third with S3. For each extraction, solvent is delivered to the cartridge outlet sensor, allowed to incubate with the sample for 10 seconds, and then transferred to the flask with argon. S2B, which is more polar than S3, improves the recovery of polar residues, particularly histidine, arginine, aspartic acid and glutamic acid. Using S3 for the first extraction reduces the possibility of polybrene/sample washout.

The argon delivery after each extraction must be long enough to transfer the contents of the cartridge to the flask and dry the cartridge outlet sensor. If there are still droplets of liquid at the outlet sensor, solvent delivery may be cut short.

PVDF Membrane-bound Samples

Samples applied to PVDF membrane, whether sequenced using gas or liquid cleavage, are extracted in the same way. PVDF membrane (blot) cycles use three extractions, the first with S3 (butyl chloride) the second with S2B (ethyl acetate) and the third with S3. For each extraction, solvent is delivered to the cartridge outlet sensor, allowed to incubate with the sample for 10 seconds, and then transferred to the flask with argon. As with samples on glass fiber, extraction with S2B, improves the recovery of polar residues. The argon delivery after each extraction must be long enough to transfer the contents of the cartridge to the flask and dry the cartridge outlet sensor. If there are still droplets of liquid at the outlet sensor, solvent delivery may be cut short.

Flask Chemistry

The ATZ-amino acid is extracted from the cartridge and transferred to the flask for conversion into the more stable PTH-amino acid derivative. In preparation for the transfer of the ATZ-amino acid to the flask, a small volume of 20% acetonitrile (S4) is delivered to the flask. The presence of the S4 in the flask reduces the modification of certain amino acid residues, in particular serine and threonine.

Pre-Conversion Drying

During transfer and immediately after transfer, the liquid in the flask is bubbled to evaporate the S3 and S2B transferred from the cartridge, and to reduce the volume of the sample prior to the addition of R4 to the flask for conversion. At this point in the conversion cycle, the sample should not be completely dried in the flask, but reduced to a volume of 10–20 µL. Completely drying the sample before conversion will cause reduced recovery of labile residues, particularly serine and threonine. To simplify optimization of this drying step, function 236, Pre-conversion Dry, has a global time setting. Changing the global time setting in the Function view will adjust the length of this step in any cycles in which it is used. See “Setting Global Time” on page 4-3 for instructions on modifying a global time setting.

Conversion

Conversion of the ATZ-amino acid into a PTH-amino acid takes place in aqueous acid medium. A large loop load of R4 is added to the flask and allowed to incubate with the sample for approximately 9 minutes. It is possible to use a small load of R4 for the

conversion instead of the large load. This would reduce the amount of drying necessary after conversion.

Post-Conversion Drying

After conversion, the sample must be completely dried to remove all the TFA which will interfere in the chromatography of the early eluting PTH-amino acids. In the standard flask cycles, the flask will appear dry 90–120 seconds before the end of the drying step which follows the Post-conversion Dry step. This will not adversely affect the recovery of the PTH-amino acids.

To simplify optimization of this drying step, function 237, Post-conversion Dry, has a global time setting. Changing the global time setting in the Function view will adjust the length of this step in any cycles in which it is used. See “Setting Global Time” on page 4-3 for instructions on modifying a global time setting.

PTH-amino Acid Solubilization

The dried PTH-amino acid in the flask is dissolved in 20% acetonitrile (S4) for subsequent transfer to the injector loop. Two large loop loads of S4 are used to dissolve the sample in the standard flask cycles. Bubbling of the flask contents assists the dissolution of the sample.

Sample Transfer and Injection

Once the sample has been reconstituted in the flask, it must be transferred to the HPLC injector loop. Transfer is accomplished by pressurizing the flask with argon and driving the sample out through the pick-up line and into the injector loop. When the sample loop load sensor detects fluid, the injector valve is switched from the load to the inject position, moving the sample into the HPLC solvent stream. The HPLC begins running the programmed gradient, and data collection is started. The following steps are necessary in a flask cycle in order for a successful sample injection:

Steps	Function #	Description
Prepare Pump	227	Gradient information is downloaded to the pump from the Procise software. After downloading is complete (30–60 seconds), the pump will start, pressurize and run at the initial gradient conditions.
Load Position	226	This function must precede the Load Injector step in order for the sample loop to be flushed before the sample in the flask is transferred into the sample loop.
Flush Injector	221	Flushes the sample loop from valve 44. Does not flush through the flask.
Load Injector	225	Activates the sample loop sensors. Transfers sample from the flask into the HPLC sample loop.

Sample Volume

The volume of sample transferred to the injector loop is determined by the size of the loop loads sent to the flask. The standard volume of a large loop load of S4 is 60 µL. Two loads to the flask provide a total sample volume of 120 µL. Bubbling in the flask reduces the acetonitrile content of the sample in the flask, reduces the sample volume, and insures proper binding of the PTH-amino acids to the HPLC column.

Injection Percentage

The standard injection percentage for the Procise Protein Sequencer is 70 to 80%. This percentage of injection was selected to provide consistent fluid detection at both the Sample Loop Load and Sample Loop Full sensors. The percentage of sample injected can be increased by lengthening the global time setting for the Concentrate Sample step (function 238) in order to reduce the total sample volume. At greater than 80% injection, intermittent failures to detect fluid may be reported to the Event Log by the Sample Loop Full Sensor. The reduced sample volume may not be enough to consistently provide a wet reading at the Sample Loop Full Sensor.

PTH Separation 7

Overview

About This Chapter

This chapter describes the procedures used for the Procise® liquid chromatography (HPLC) system. Instructions for preparing solvents and optimizing the separation of PTH amino acids are in this section. Routine operations of the Procise HPLC system are controlled by Procise control software. For complete descriptions of the menus used to control the pumps, refer to the *Model 140C Micro gradient Delivery System User's Manual.*

In This Chapter

This chapter contains the following topics:

Amino Acid Table

Abbreviations Table

Table 7-1 Amino Acid Abbreviations and Symbols

Amino Acid	Abbreviation	Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Glutamine	Gln	Q
Glutamic Acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

HPLC Solvents

About the HPLC Solutions

See “Chemical Warnings Table” on page 1-7 for warnings about A3, B2, acetonitrile, or the premix buffer concentrate solutions.

Solvent A3

The composition of Solvent A3 is 3.5% tetrahydrofuran/water. Together with the Premix buffer concentrate, Solvent A3 provides optimal separation of the PTH-amino acids.

Solvent B2

Solvent B2 contains 12% isopropanol in acetonitrile to resolve PTH-Trp from the chemical artifact, diphenylurea (DPU).

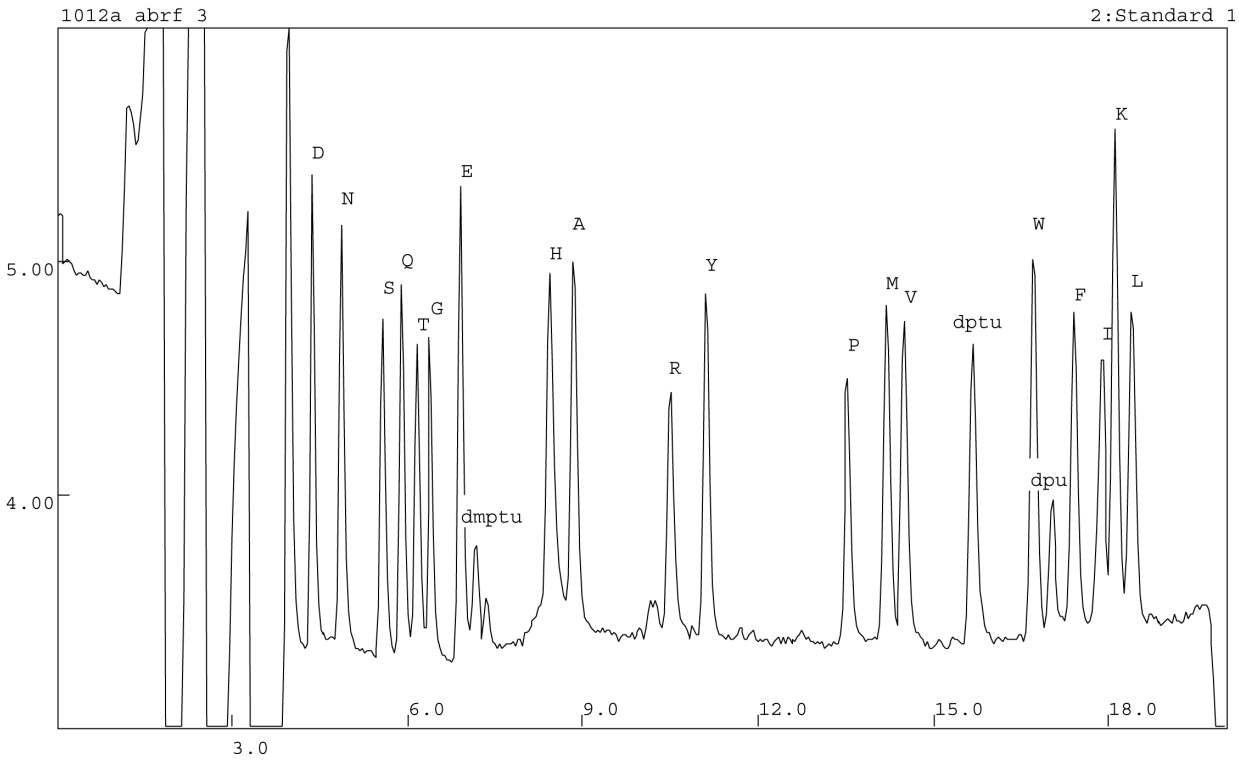


Figure 7-1 5 pmoles of PTH-amino acid standard was analyzed using solvent B2 and solvent A3 containing Premix Buffer Concentrate. Notice that the DPU is favorably positioned between Trp and Phe.

Premix Buffer Concentrate

The premix buffer employs an ion-pairing additive to improve both peak shape and retention time reproducibility for the PTH-derivatives of histidine, arginine and the pyridylethyl derivative of cysteine. PTH-derivatives with positively-charged side-chain groups interact with underivatized silanol groups on the silica particles in a column causing peak broadening and retention time shifting. By adding an ion-pairing modifier to the mobile phase, the interaction of the basic derivatives with free silanol is significantly reduced through preferential interaction with a strongly acidic ion-pairing additive.

Adding Premix Buffer

Influence the separation by adding Premix buffer.

If...	Then...
the desired elution order is: PTH-His before PTH-Ala, PTH-Arg before PTH-Tyr, and PTH-PE-Cys before PTH-Pro	add ~25 mL of Premix buffer concentrate (P/N 401446) to 1 liter of solvent A3, 3.5% THF(P/N 401464). Cap and mix well.
the PE-Cys is not a derivative of interest	it is also possible to position His after Ala and Arg after Tyr by using less Premix buffer. Approximately 10 mL of Premix will usually give good separation with His and Arg in these later elution positions (see Figure 7-2).

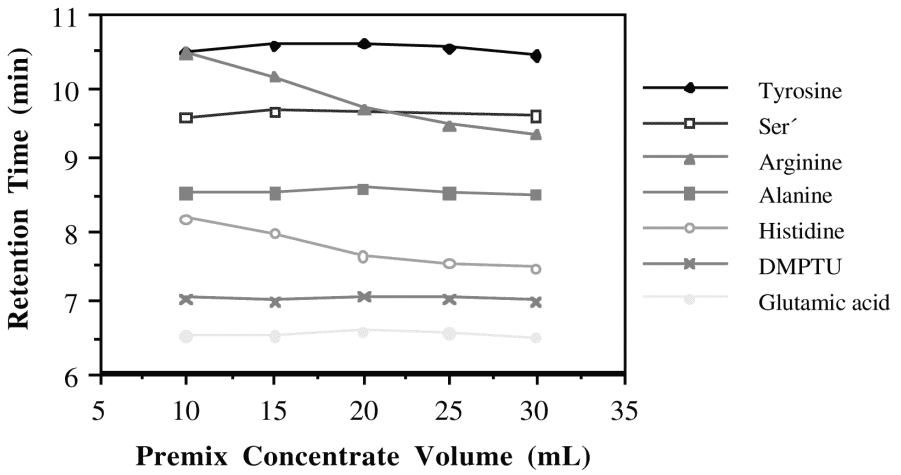


Figure 7-2 Effect of Premix Buffer concentration on retention time.

Gradient and Programming

Fast Gradient

The fast gradient is programmed in the Procise software. It provides reduced chromatographic turnaround time through increased mobile phase flow rate. The gradient programmed in Procise software is the linear gradient which provides flattened baseline throughout the chromatography. However, any fast gradients published previously in User Bulletins or User's Manuals should provide satisfactory separation with this buffer system.

Table 7-2 Fast Gradient Conditions

Topic	Description
Target Pressure	1000 psi
Target Time	1.0 minutes
Pressure Limits	0–4000 psi

Step#	Time (min.)	%B	Flow rate (µL/min.)	Events
1	0	6	325	12
2	0.3	6	325	1
3	0.4	16	325	1
4	18	45	325	1
5	18.5	90	325	1
6	21.5	90	325	1
7	22	50	325	1

Separating PTH-derivatives Gln, Thr and Gly

Adding a gradient step at 0.4 minutes and increasing the solvent B concentration 3–5% over initial concentration can provide improvement in the separation of the PTH-derivatives of Gln, Thr and Gly. This modification to the gradient allows the initial% B to be lower in order to improve the retention of PTH-Asp on the column without compromising the separation of DMPTU from PTH-Glu.

Changing an Active Gradient

The gradients programmed in Procise software can be edited without having to first save them under another name. The active gradient can be changed during a run and the altered gradient will be downloaded to the 140C pump.

To make changes to an active gradient:

Step	Action
1	Select the Gradient screen and select gradient Fast Normal 1 from the pull-down list.
2	Highlight the time line that requires the change, and alter the value in the Time box and/or %B box at the top of the column.
3	To insert or delete row, highlight the time line and click the Insert Row or Delete Row button. Alter the time and %B as desired.
4	To save the changes, pull down the File menu from the top menu bar and select Save Gradient

IMPORTANT By selecting Save Gradient from the File menu, the current fast gradient will be altered and the changes sent to the sequencer if it is active. To save the new gradient without modifying the original fast gradient, select Save As… from the File menu and enter the new gradient name.

Optimizing the Chromatography

Flattening the Baseline

In order to achieve a high sensitivity sequence, it is critical to minimize any baseline rise. One factor which causes the baseline rise in PTH chromatograms, is the slightly higher absorbance properties of solvent B2. Eliminating this problem can increase accuracy in computer integration at high sensitivity.

Acetone has a very high UV absorbance at 269 nm, a wavelength that is optimal for PTH amino acid analysis. When small amounts of acetone are added to the solvent A3 and a linear gradient is implemented, the absorbance of solvent A3 and solvent B2 will match, therefore most of the baseline rise will be eliminated. See “Chemical Warnings Table” on page 1-7 for warnings about B2, A3, and acetone.

Use the following procedure to prepare the buffer:

To flatten the baseline by adding acetone to Solvent A3:

Step	Action
1	Make 1% acetone/H_2O solution: Mix 1 mL of HPLC grade acetone and 99 mL of milli-Q H_2O in a 100 mL clean bottle.
2	Add 1 mL of 1% acetone/H_2O to 1 liter of solvent A3 buffer. Mix well.

Reducing Negative Baseline Slope at the Start of the Chromatogram

Some HPLC and/or PTH-columns may exhibit a negative slope in the baseline from DTT to Glu before flattening out in the latter part of the chromatogram. The addition of a small amount of phosphate ion to Solvent A usually reduces or eliminates this problem. See “Chemical Warnings Table” on page 1-7 for warnings about sodium phosphate.

To reduce a negative baseline by adding phosphate ion to Solvent A:

Step	Action
1	Prepare a 1.0 M stock solution of NaH_2PO_4 or KH_2PO_4 (monobasic sodium or potassium phosphate, sodium or potassium dihydrogen phosphate).
2	Add 100 µL of the phosphate solution to 1 L of Solvent A to provide a final concentration of 0.1 mM phosphate. **Note** This will flatten the baseline over several cycles and prevent reappearance of the slope.

Optimizing the PTH-AA Separation

PTH-AA Separation at Installation

The steps for optimizing the separation of specific PTH-amino acids and chemistry artifacts are described in this section.

During installation, the PTH-amino acid separation is optimized for the PTH column supplied with your instrument. The gradient used is stored in the Procise control software. These conditions serve as the starting point. To maintain optimum separation, the gradient may require fine-tuning as the column ages.

Positioning the Positively Charged PTH-AAs

The positively charged PTH-AAs, are:

- Histidine
- Arginine
- Pyridylethyl cysteine

Increasing the ionic strength of the mobile phase reduces the retention of the basic derivatives on the column. Suggested elution positions for the basic derivatives are His between DMPTU and Ala, Arg between Ser´ and Tyr, and PECys before Pro.

For the majority of columns, these elution positions can be obtained by using ~20 mL of the Premix buffer concentrate per liter of solvent A3, and making minor gradient modification. However, increasing the buffer concentration can be a useful method to make Arg elute earlier than Ser´ and to make PECys elute earlier than Pro. See “Chemical Warnings Table” on page 1-7 for warnings about A3, and premix buffer concentrate solutions.

If...	Then...
PECys is not a derivative of interest	it is possible to position His after Ala and Arg after Tyr by using less Premix buffer. Approximately 10 mL of Premix will usually give a good separation with His and Arg in these later elution positions.

Histidine

If...	Then...
His coelutes with Ala	increase the buffer concentration.
His needs to move before Ala	add an additional 5 mL of Premix buffer concentrate per liter of Solvent A3.

Arginine

If...	Then...
Arg coelutes with Tyr	increase the buffer concentration.
Arg needs to move before Tyr	a. add an additional 5 mL of Premix buffer concentrate per liter of solvent A3. b. Increase the %B at 0.4 minutes to 14–16%.

If...	Then...
the Ser´ coelutes with the Arg peak	improve the separation by shifting the Ser´ peak before Arg by: a. Decreasing the column temperature 2–5 °C. Adjust the temperature carefully because the separation of Met from Val is reduced by decreasing the column temperature. b. Decreasing the flow rate. A decrease in flow rate from 325 mL/min–300 mL/min moves Arg slightly relative to Ser´ without significantly impacting any other separations. The %B at 18 minutes should be decreased by 1% to maintain optimum separation of Ile/Lys.

Pyridylethyl Cysteine

If...	Then...
PECys coelutes with Pro	increase the buffer concentration. Add approximately 5 mL of Premix buffer concentrate per liter of Solvent A3 to move PECys before Pro.

The Acidic Amino Acids

The acidic amino acids are:

- Aspartic acid
- Glutamic acid

Aspartic Acid

If...	Then...
Asp needs to be separated from the DTT peak	decrease the initial %B. If the initial %B is reduced below 8%, DMPTU will probably move under Glu. (The DTT peak elutes immediately after the negative dip of the injection artifact.) Add a gradient step at 0.4 minutes.
decreasing the initial %B causes Glu to coelute with DMPTU	add a gradient step at 0.4 minutes and set the %B to 14–16%. The initial %B can then be lowered below 5% without losing the Glu/DMPTU separation. **Note** Decreasing the initial %B may cause Asp and phenylthiourea (PTU, a reaction product of PITC and NH_3) to coelute.
the gradient adjustments fail to separate Asp from the DTT peak	add 100 µL of neat TFA per liter of Solvent A.

If...	Then...
Asp needs to be separated from phenylthiourea (PTU, reaction product of PITC and NH 3)	a. Increase initial %B. Increasing the initial %B to 8–10% will usually separate the Asp peak before PTU, moving it towards the DTT peak. (The DTT peak elutes immediately after the negative dip of the injection artifact.) b. Decrease the flow rate. A decrease in flow rate from 325 mL/min–300 mL/min will move Asp away from PTU without significantly impacting any other separation. The %B at 18 minutes should be decreased by 1% to maintain optimum separation of Ile/Lys.

Glutamic Acid

If...	Then...
Glu needs to be separated from DMPTU	increase the initial %B. If the Asp/DTT peak separation will not be compromised, increase the initial %B by 2–3%. add a gradient step at 0.4 minutes. If increasing the initial %B will cause the Asp to elute too early, add a gradient step at 0.4 minutes and set the %B to 14–16%.

Improving the Separation of Other Amino Acids

To improve the...	Then...
Met/Val separation	increase the column oven temperature. Increase the temperature in 2 °C increments. Do not raise the temperature above 59 °C.
Ile/Lys separation	decrease the %B at 18 minutes If the peaks are more than 50% separated, decrease the %B by 1%. If the peaks are less than 50% separated, decrease the %B by 2%.
Lys/Leu separation	increase the %B at 18 minutes. If the peaks are more than 50% separated, increase the %B by 1%. If the peaks are less than 50% separated, increase the %B by 2%.

Optimization Table

In Table 7-3, the arrow above an amino acid indicates the direction in which the peak moves after changing the variable listed in the column. Left is toward the injection point.

Table 7-3 Optimization Guidelines

Variable	Major Effect	Minor Effect
Increase initial %B	← ← DTT D PTU	← ← ← E DMPTU H; S´ R
Decrease initial %B	→ → DTT D PTU	← → → → S Q E DMPTU H;
Increase initial %B at 0.4 minutes	← ← ← E DMPTU H; S´ R;	← S Q T
Decrease %B at 0.4 minutes	→ → E DMPTU H	
Increase final %B at 10 and 18 minutes	← I K L	
Decrease final %B at 10 and 18 minutes	→ I K L	
Increase column temperature (2 °C)	← → M V	← ← ← H R PECys
Decrease column temperature (2 °C)	→ S´ R;	→ T G
Increase molarity	← ← H A; R S´;	← PECys P
Fast Gradients Only: Decrease flow rate	→ → D PTU; S´ R	

Function Listing

A

Overview

About This Appendix

This appendix lists all the function numbers from 1–450 and the valves and reagents associated with the function.

In This Appendix

This appendix contains the following topic:

Function List Table

Function List The table below lists the assigned number, reagent, and the valves involved in a function.

Number	Reagent	Name	Valves	Global Value	Sensor Function	Global Time
001	R1	Del R1, Cart (top)	6, 7, 17, 18, 19, 20, 34, 35, 36, 37, 40	0	N	Y
002	R1	Del R1, Cart (bottom)	6, 11, 10, 17, 18, 19, 20, 34, 35, 36, 37, 16	0	N	Y
003	R1	Del R1, Cart (sensor)	6, 7, 17, 18, 19, 20, 34, 35, 36, 37, 40	0	Y	Y
004	R1	Del R1, Waste	6, 1	0	N	N
005	R1	Load R1, Cart (sm loop)	6, 7, 22	0	Y	Y
006	R1	Load R1, Cart (lg loop)	6, 7, 21	0	Y	Y
007	R1	Vent R1	55	0	N	N
008	R1	Flush R1	55	0	N	Y
009	R1	Backflush R1	6, 11, 55, 15	0	N	N
010	R1	Reserved		0	N	N
011	R2g	Del R2g, Cart (top)	3, 7, 17, 18, 19, 20, 34, 35, 36, 37, 40	0	N	Y
012	R2g	Del R2g, Cart (bottom)	3, 11, 10, 17, 18, 19, 20, 34, 35, 36, 37, 16	0	N	Y
013	R2g	Not Available		0	N	N
014	R2g	Del R2g, Waste	3, 1	0	N	N
015	R2g	Not Available		0	N	N
016	R2g	Not Available		0	N	N
017	R2g	Vent R2g	58	0	N	N
018	R2g	Flush R2g	58	0	N	Y
019	R2g	Backflush R2g	3, 11, 58, 15	0	N	N
020	R2g	Reserved		0	N	N
021	R3	Del R3, Cart (top)	8, 23, 17, 18, 19, 20, 34, 35, 36, 37, 40	0	N	Y
022	R3	Del R3, Cart (bottom)	8, 10, 17, 18, 19, 20, 34, 35, 36, 37, 16	0	N	Y
023	R3	Del R3, Cart (sensor)	8, 23, 17, 18, 19, 20, 34, 35, 36, 37, 40	0	Y	Y
024	R3	Del R3, Waste	8, 23, 16	0	N	N
025	R3	Load R3, Cart (sm loop)	8, 23, 22	0	Y	Y
026	R3	Load R3, Cart (lg loop)	8, 23, 21	0	Y	Y
027	R3	Vent R3	53	0	N	N
028	R3	Flush R3	53	0	N	Y
029	R3	Backflush R3	8, 53, 15	0	N	N
030	R3	Transfer R3, Cart (gas)	7, 11, 15, 17, 18, 19, 20, 34, 35, 36, 37, 40	0	N	N
031	R3g	Del R3g, Cart (top)	9, 23, 17, 18, 19, 20, 34, 35, 36, 37, 40	0	N	Y
032	R3g	Del R3g, Cart (bottom)	9, 10, 17, 18, 19, 20, 34, 35, 36, 37, 16	0	N	Y
033	R3g	Not Available		0	N	N
034	R3g	Del R3g, Waste	9, 23, 16	0	N	N
035	R3g	Not Available		0	N	N
036	R3g	Not Available		0	N	N
037	R3g	Vent R3g	53	0	N	N

Number	Reagent	Name	Valves	Global Value	Sensor Function	Global Time
038	R3g	Flush R3g	53	0	N	Y
039	R3g	Backflush R3g	9, 53, 15	0	N	N
040	R3g	Reserved		0	N	N
041	S1	Del S1, Cart (top)	14, 23, 17, 18, 19, 20, 34, 35, 36, 37, 40	0	N	Y
042	S1	Del S1, Cart (bottom)	14, 10, 17, 18, 19, 20, 34, 35, 36, 37, 16	0	N	Y
043	S1	Del S1, Cart (sensor)	14, 23, 17, 18, 19, 20, 34, 35, 36, 37, 40	0	Y	Y
044	S1	Del S1, Waste	14, 23, 16	0	N	N
045	S1	Load S1, Cart (sm loop)	7, 11, 14, 22	0	Y	Y
046	S1	Load S1, Cart (lg loop)	7, 11, 14, 21	0	Y	Y
047	S1	Vent S1	56	0	N	N
048	S1	Flush S1	56	0	N	Y
049	S1	Backflush S1	14, 56, 15	0	N	N
050	S1	Reserved		0	N	N
051	S2	Del S2, Cart (top)	12, 23, 17, 18, 19, 20, 34, 35, 36, 37, 40,	0	N	Y
052	S2	Del S2, Cart (bottom)	12, 10, 17, 18, 19, 20, 34, 35, 36, 37, 16	0	N	Y
053	S2	Del S2, Cart (sensor)	12, 23, 17, 18, 19, 20, 34, 35, 36, 37, 40	0	Y	Y
054	S2	Del S2, Waste	12, 23, 16	0	N	N
055	S2	Load S2, Cart (sm loop)	7, 11, 12, 22	0	Y	Y
056	S2	Load S2, Cart (lg loop)	7, 11, 12, 21	0	Y	Y
057	S2	Vent S2	54	0	N	N
058	S2	Flush S2	54	0	N	Y
059	S2	Backflush S2	12, 54, 15	0	N	N
060	S2	Reserved		0	N	N
061	S3	Del S3, Cart (top)	13, 23, 17, 18, 19, 20, 34, 35, 36, 37, 40	0	N	N
062	S3	Del S3, Cart (bottom)	13, 10, 17, 18, 19, 20, 34, 35, 36, 37, 16	0	N	Y
063	S3	Del S3, Cart (sensor)	13, 23, 17, 18, 19, 20, 34, 35, 36, 37, 40	0	Y	Y
064	S3	Del S3, Waste	13, 23, 16	0	N	N
065	S3	Load S3, Cart (sm loop)	7, 11, 13, 22	0	Y	Y
066	S3	Load S3, Cart (lg loop)	7, 11, 13, 21	0	Y	Y
067	S3	Vent S3	52	0	N	N
068	S3	Flush S3	52	0	N	Y
069	S3	Backflush S3	13, 52, 15	0	N	N
070	S3	Reserved		0	N	N
071	X1	Del X1, Cart (top)	5, 7, 17, 18, 19, 20, 34, 35, 36, 37, 40	0	N	Y
072	X1	Del X1, Cart (bottom)	5, 11, 10, 17, 18, 19, 20, 34, 35, 36, 37, 16	0	N	Y
073	X1	Del X1, Cart (sensor)	5, 7, 17, 18, 19, 20, 34, 35, 36, 37, 40	0	Y	Y
074	X1	Del X1, Waste	5, 1	0	N	N
075	X1	Load X1, Cart (sm loop)	5, 7, 22	70	Y	Y
076	X1	Load X1, Cart (lg loop)	5, 7, 21	0	Y	Y
077	X1	Vent X1	59	0	N	N
078	X1	Flush X1	59	0	N	Y

Number	Reagent	Name	Valves	Global Value	Sensor Function	Global Time
079	X1	Backflush X1	5, 11, 59, 15	0	N	N
080	X1	Reserved		0	N	N
081	X1g	Del X1g, Cart (top)	2, 7, 17, 18, 19, 20, 34, 35, 36, 37, 40	0	N	Y
082	X1g	Del X1g, Cart (bottom)	2, 11, 10, 17, 18, 19, 20, 34, 35, 36, 37, 16	0	N	Y
083	X1g	Not Available		0	N	N
084	X1g	Del X1g, Waste	2, 1	0	N	N
085	X1g	Not Available		0	N	N
086	X1g	Not Available		0	N	N
087	X1g	Vent X1g	59	0	N	N
088	X1g	Flush X1g	59	0	N	Y
089	X1g	Backflush X1g	2, 11, 59, 15	0	N	N
090	X1g	Reserved		0	N	N
091	X3	Del X3, Cart (top)	4, 7, 17, 18, 19, 20, 34, 35, 36, 37, 40	0	N	Y
092	X3	Del X3, Cart (bottom)	4, 11, 10, 17, 18, 19, 20, 34, 35, 36, 37, 16	0	N	Y
093	X3	Del X3, Cart (sensor)	4, 7, 17, 18, 19, 20, 34, 35, 36, 37, 40	0	Y	Y
094	X3	Del X3, Waste	4, 1	0	N	N
095	X3	Load X3, Cart (sm loop)	4, 7, 22	0	Y	Y
096	X3	Load X3, Cart (lg loop)	4, 7, 21	0	Y	Y
097	X3	Vent X3, Cart	60	0	N	N
098	X3	Flush X3, Cart	60	0	N	Y
099	X3	Backflush X3, Cart	4, 11, 60, 15	0	N	N
100	X3	Reserved		0	N	N
101	S1	Wash Input Block (S1)	7, 11, 14, 16	0	N	Y
102	S1	Wash Output Block (S1)	14, 10, 40	0	N	Y
103	S1	Transfer to Flask (S1)	14,23,17,18,19,20,34,35,36,37,38,45	0	Y	Y
104	S1	Transfer to FC (S1)	14,23,17,18,19,20,34,35,36,37,39	0	N	Y
105	S1	Reserved		0	N	N
106	S2	Wash Input Block (S2)	7, 11, 12, 16	0	N	Y
107	S2	Wash Output Block (S2)	12, 10, 40	0	N	Y
108	S2	Transfer to Flask (S2)	12, 23, 17, 18, 19, 20, 34, 35, 36, 37, 38, 45	0	Y	Y
109	S2	Transfer to FC (S2)	12, 23, 17, 18, 19, 20, 34, 35, 36, 37, 39	0	N	Y
110	S2	Reserved		0	N	N
111	S3	Wash Input Block (S3)	7, 11, 13, 16	0	N	Y
112	S3	Wash Output Block (S3)	13, 10, 40	0	N	Y
113	S3	Transfer to Flask (S3)	13, 23, 17, 18, 19, 20, 34, 35, 36, 37, 38, 45	0	Y	Y
114	S3	Transfer to FC (S3)	13, 23, 17, 18, 19, 20, 34, 35, 36, 37, 39	0	N	Y
115	S3	Reserved		0	N	N
116	X3	Wash Input Block (X3)	4, 7, 16	0	N	Y
117	X3	Wash Output Block (X3)	4, 11, 10, 40	0	N	Y
118	X3	Transfer to Flask (X3)	4, 7, 17, 18, 19, 20, 34, 35, 36, 37, 38, 45	0	Y	Y

Number	Reagent	Name	Valves	Global Value	Sensor Function	Global Time
119	X3	Transfer to FC (X3)	4, 7, 17, 18, 19, 20, 34, 35, 36, 37, 39	0	N	Y
120	X3	Reserved		0	N	N
121		Transfer to Flask (gas)	15, 23, 17, 18, 19, 20, 34, 35, 36, 37, 38, 45	0	Y	Y
122		Transfer to FC (gas)	15, 23, 17, 18, 19, 20, 34, 35, 36, 37, 39	0	N	Y
123		Select Cartridge A		0	N	N
124		Select Cartridge B		0	N	N
125		Select Cartridge C		0	N	N
126		Select Cartridge D		0	N	N
127		Ready Transfer to Flask		0	N	N
128		Transfer Complete		0	N	N
129		Pressurize Cart, top	23, 17, 18, 19, 20, 15	0	N	Y
130		Pressurize Cart, bottom	10, 34, 35, 36, 37, 15	0	N	Y
131		Dry Cart (top)	23, 17, 18, 19, 20, 34, 35, 36, 37, 40, 15	0	N	Y
132		Dry Cart (bottom)	10, 17, 18, 19, 20, 34, 35, 36, 37, 16, 15	0	N	Y
133		Dry Cart (high, top)	23, 17, 18, 19, 20, 34, 35, 36, 37, 15, 40, 46	0	N	Y
134		Dry Cart (high, bottom)	10, 17, 18, 19, 20, 34, 35, 36, 37, 16, 15, 46	0	N	Y
135		Flush Cart Reagent Block	1, 11, 15, 46	0	N	Y
136		Flush Cart Solvent Block	15, 16, 23, 46	0	N	Y
137		Flush Input Block	7, 11, 15, 16, 46	0	N	Y
138		Flush Output Block	10, 15, 40, 46	0	N	Y
139		Flush Small Loop (Cart)	7, 11, 15, 22, 46	0	N	Y
140		Flush Large Loop (Cart)	7, 11, 15, 21, 46	0	N	Y
141		Flush Transfer Line	15, 10, 38, 45	0	N	Y
142		Set Cart Temperature		0	N	N
143		Wash Cart Reagent Block	1, 11, 12	0	N	N
144		Wash Cart Solvent Block	12, 16, 23	0	N	N
145		Wash Small Loop (Cart)	7, 11, 12, 22	0	N	N
146		Wash Large Loop (Cart)	7, 11, 12, 21	0	N	N
147		End Cartridge Select		0	N	N
148		Cartridge Wait	17,18,19,20,34,35,36,37	0	N	Y
149	S2	Wash Transfer Line (S2)	10, 12, 38, 45	0	N	Y
150		Reserved		0	N	N
151	R4	Del R4, Flask	28, 32, 45	0	N	Y
152	R4	Load R4, Flask (sm loop)	28, 30	0	Y	Y
153	R4	Load R4, Flask (lg loop)	28, 31	0	Y	Y

Number	Reagent	Name	Valves	Global Value	Sensor Function	Global Time
154	R4	Vent R4	51	0	N	Y
155	R4	Flush R4	51	0	N	Y
156	R4	Backflush R4	28, 51, 24	0	N	Y
157	R4	Del R4, Waste	28, 31	0	N	N
158	R4	Reserved		0	N	N
159	R4	Reserved		0	N	N
160	R4	Reserved		0	N	N
161	R5	Del R5, Flask	29, 32, 45	0	N	Y
162	R5	Load R5, Flask (sm loop)	29, 30	0	Y	Y
163	R5	Load R5, Flask (lg loop)	29, 31	0	Y	Y
164	R5	Vent R5	49	0	N	Y
165	R5	Flush R5	49	0	N	Y
166	R5	Backflush R5	29, 49, 24	0	N	Y
167	R5	Del R5, Waste	29, 31	0	N	N
168	R5	Reserved		0	N	N
169	R5	Reserved		0	N	N
170	R5	Reserved		0	N	N
171	S4	Del S4, Flask	25, 32, 45	0	N	Y
172	S4	Load S4, Flask (sm loop)	25, 30	0	Y	Y
173	S4	Load S4, Flask (lg loop)	25, 31	0	Y	Y
174	S4	Vent S4	50	0	N	Y
175	S4	Flush S4	50	0	N	Y
176	S4	Backflush S4	25, 50, 24	0	N	Y
177	S4	Del S4, Waste	25, 31	0	N	N
178	S4	Reserved		0	N	N
179	S4	Reserved		0	N	N
180	S4	Reserved		0	N	N
181	X2	Del X2, Flask	27, 32, 45	0	N	Y
182	X2	Load X2, Flask (sm loop)	27, 30	0	Y	Y
183	X2	Load X2, Flask (lg loop)	27, 31,	0	Y	Y
184	X2	Vent X2	57	0	N	Y
185	X2	Flush X2	57	0	N	Y
186	X2	Backflush X2	27, 57, 24	0	N	Y
187	X2	Del X2, Waste	27, 31	0	N	N
188	X2	Reserved		0	N	N
189	X2	Reserved		0	N	N
190	X2	Reserved		0	N	N
191	X2g	Del X2g, Flask	33, 32, 45	0	N	Y
192	X2g	Not Available		0	N	N

Number	Reagent	Name	Valves	Global Value	Sensor Function	Global Time
193	X2g	Not Available		0	N	N
194	X2g	Vent X2g	57	0	N	Y
195	X2g	Flush X2g	57	0	N	Y
196	X2g	Backflush X2g	33, 57, 24	0	N	Y
197	X2g	Del X2g, Waste	31, 33	0	N	N
198	X2g	Reserved		0	N	N
199	X2g	Reserved		0	N	N
200	X2g	Reserved		0	N	N
201	X3	Del X3, Flask	26, 32, 45	0	N	Y
202	X3	Load X3, Flask (sm loop)	26, 30	0	Y	Y
203	X3	Load X3, Flask (lg loop)	26, 31	0	Y	Y
204	X3	Vent X3, Flask	60	0	N	Y
205	X3	Flush X3, Flask	60	0	N	Y
206	X3	Backflush X3, Flask	26, 60, 24	0	N	Y
207	X3	Del X3, Waste, Flask	26, 31	0	N	N
208	X3	Reserved		0	N	N
209	X3	Reserved		0	N	N
210	X3	Reserved		0	N	N
211		Bubble Flask (h press)	41, 44, 45, 48	0	N	Y
212		Bubble Flask	41, 44, 45	0	N	Y
213		Dry Flask	24, 32, 41, 44, 45	0	N	Y
214		Dry Flask (h press)	24, 32, 41, 44, 45, 48	0	N	Y
215		Empty Flask	24, 32, 41, 43	0	N	Y
216		Empty Flask (l press)	24, 32, 41, 43, 47	0	N	Y
217		Flush Small Loop (Flask)	24, 30	0	N	Y
218		Flush Large Loop (Flask)	24, 31	0	N	Y
219		Wash Small Loop (Flask)	25, 30	0	N	N
220		Wash Large Loop (Flask)	25, 31	0	N	N
221		Flush Injector	42, 44, 48	0	N	Y
222		Flush Flask/Injector	24, 32, 41, 42	0	N	Y
223		Inject Position		0	N	Y
224		Flush Injector (Low Pres)	42, 44	0	N	Y
225		Load Injector	24, 32, 41, 42, 47	0	Y	Y
226		Load Position		0	N	Y
227		Prepare Pump		0	N	Y
228		Ready to Receive	41, 44, 45	0	N	Y

Number	Reagent	Name	Valves	Global Value	Sensor Function	Global Time
229		Set Column Temperature		0	N	Y
230		Set Flask Temperature		0	N	Y
231		Stop Pump		0	N	Y
232		Start Gradient		0	N	N
233		Set as Blank Cycle		0	N	N
234		Set as Standard Cycle		0	N	N
235		Set as Residue Cycle		0	N	N
236		Pre-Conversion Dry 2	4, 32, 41, 44, 45	80	N	Y
237		Post-Conversion Dry	24, 32, 41, 44, 45	200	N	Y
238		Concentrate Sample	24, 32, 41, 44, 45	15	N	Y
239		Reserved		0	N	N
240		Reserved		0	N	N
241		Reserved		0	N	N
242		Reserved		0	N	N
243		Reserved		0	N	N
244		Reserved		0	N	N
245		Reserved		0	N	N
246		Reserved		0	N	N
247		Reserved		0	N	N
248		Reserved		0	N	N
249		Reserved		0	N	N
250		Reserved		0	N	N
251		490A Relay 1 Off		0	N	Y
252		490A Relay 1 On		0	N	Y
253		490A Relay 1 Pulse		0	N	Y
254		490A Relay 2 Off		0	N	Y
255		490A Relay 2 On		0	N	Y
256		490A Relay 2 Pulse		0	N	Y
257		Wait		0	N	Y
258		Begin		0	N	N
259		End		0	N	N
260		Pause for Bottle Change		0	N	N
261		Set for Bottle R1		0	N	N
262		Set for Bottle R2		0	N	N
263		Set for Bottle R3		0	N	N
264		Set for Bottle R4		0	N	N
265		Set for Bottle R5		0	N	N
266		Set for Bottle S1		0	N	N
267		Set for Bottle S2		0	N	N
268		Set for Bottle S3		0	N	N

Number	Reagent	Name	Valves	Global Value	Sensor Function	Global Time
269		Set for Bottle S4		0	N	N
270		Set for Bottle X1		0	N	N
271		Set for Bottle X2		0	N	N
272		Set for Bottle X3		0	N	N
273		Init Sm Loop Snsr, Cart		0	Y	N
274		Init Lg Loop Snsr, Cart		0	Y	N
275		Init Cart A Snsr		0	Y	N
276		Init Cart B Snsr		0	Y	N
277		Init Cart C Snsr		0	Y	N
278		Init Cart D Snsr		0	Y	N
279		Init Transfer Snsr		0	Y	N
280		Init Sm Loop Snsr, Flask		0	Y	N
281		Init Lg Loop Snsr, Flask		0	Y	N
282		Init Injector Load Snsr		0	Y	N
283		Init Injector Full Snsr		0	Y	N
284		Open Valves	11,15 11, 15	0	N	N
285		Injector Sim Load	24, 32, 41, 42, 47	0	N	N
286		X3 to R2	3, 4, 58	0	N	N
287		X3 to R3g	4, 9, 11, 53	0	N	N
288		X3 to X1g	2, 4, 59	0	N	N
289		X3 to X2g	26, 33, 57	0	N	N
290		Vent 16 Test	7, 11, 15, 16	0	N	N
291		Vent 30 Test	24, 30, 47	0	N	N
292		Vent 43 Test	43, 44	0	N	N
293		Open Valves 15,23	15, 23	0	N	N
294		Open Valve 24	24	0	N	N
295		Open Valves 24,32	24, 32	0	N	N
296		Open Valves 24,32,45	24,32,45	0	N	N
297		Flask Out Test	44	0	N	N
298		Flask Reag Blk, Hi Test	24, 47	0	N	N
299		Open Valves 49,57,59	49, 57, 59	0	N	N
300		Waste Test	43, 44	0	N	N
301		Pause		0	N	N
302		Use Valves of Function		1	N	N
303		Select Regulator		1	N	N
304		Save Regulator Setpoint		0	N	N
305		Set Reg Setpoint (10th psi)		0	N	N
306		Wait With Valves On		0	N	N

Number	Reagent	Name	Valves	Global Value	Sensor Function	Global Time
307		Compare Pressures (10th psi)		0	N	N
308		Close Pressure Valve		0	N	N
309		Restore Reg Setpoint		0	N	N
310		Set Tolerance (100th psi)		0	N	N
311		Test Valves		0	N	N
312		Test Heaters		0	N	N
313		Test Pressure Board		0	N	N
314		Test 12-Bit A/D		0	N	N
315		Test 24-Bit A/D		0	N	N
316		Test Rheodyne		0	N	N
317		Save Regulator Pressure		0	N	N
318		Compare Saved Pressure		0	N	N
319		Compare HP Inlet (10th psi)		0	N	N
320		Select Heater		1	N	N
321		Save Heater Setpoint		0	N	N
322		Restore Heater Setpoint		0	N	N
323		Inc. Heater Setpoint (°C)		0	N	N
324		Dec. Heater Setpoint (°C)		0	N	N
325		Set Heater Tolerance (100th °C)		0	N	
326		Compare Temperatures		0	N	N
327		Reset Vacuum On Count		0	N	N
328		Log Vacuum On Count		0	N	N
329		Set Flow Meter Tolerance(SCCM)		0	N	
330		Compare Flow Meter (SCCM)		0	N	N
331		Tare Sartorius		0	N	N
332		Log Weight		0	N	N
333		X3 to R1	4, 6, 55	0	N	N
334		X3 to R3	4, 8, 11, 53	0	N	N
335		X3 to R4	26, 28, 51	0	N	N
336		X3 to R5	26, 29, 49	0	N	N
337		X3 to S1	4, 11, 14, 56	0	N	N
338		X3 to S2	4, 11, 12, 54	0	N	N
339		X3 to S3	4, 11, 13, 52	0	N	N

Number	Reagent	Name	Valves	Global Value	Sensor Function	Global Time
340		X3 to S4	25, 26, 50	0	N	N
341		X3 to X1	4, 5, 59	0	N	N
342		X3 to X2	26, 27, 57	0	N	N
343		X3 to Cart A (bottom)	4, 10, 11, 16, 17, 34	0	N	N
344		Open Valves 7,11,15,16	7, 11, 15, 16	0	N	N
345		Open Valves 1,11,15	1, 11, 15	0	N	N
346		Open Valves 1,11	1, 11	0	N	N
347		Open Valves 1,15,16,40	1, 15, 16, 40	0	N	N
348		Open Valves 11,15,16	11, 15, 16	0	N	N
349		Open Valves 15,23	15, 23	0	N	N
350		Open Valves 10,15,45	10, 15, 45	0	N	N
351		Open Valve 30	30	0	N	N
352		Flask Reag Blk Test	24	0	N	N
353		Open Valves 24,45	24, 45	0	N	N
354		Open Valve 43	43	0	N	N
355		Open Valves 44,45	44, 45	0	N	N
356 - 400		Reserved		0	N	N
401 - 450		User Functions 1 - 50		0	N	N

Cycle, Method, and Gradient Listings

B

Overview

About This Appendix

Tables of cycles, methods, and gradients are in this appendix.

In This Appendix

This appendix contains the following topics:

Cartridge Cycle List

Cart Begin Total run time: 33:20

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	146	Wash Large Loop (Cart)	10	N
3	140	Flush Large Loop (Cart)	40	N
4	144	Wash Cart Solvent Block	10	N
5	106	Wash Input Block (S2)	10	N
6	111	Wash Input Block (S3)	10	N
7	137	Flush Input Block	40	N
8	136	Flush Cart Solvent Block	40	N
9	131	Dry Cart (top)	60	N
10	140	Flush Large Loop (Cart)	10	N
11	26	Load R3, Cart (lg loop)	50	N
12	30	Transfer R3, Cart (gas)	5	N
13	136	Flush Cart Solvent Block	10	N
14	140	Flush Large Loop (Cart)	10	N
15	138	Flush Output Block	10	N
16	144	Wash Cart Solvent Block	10	N
17	143	Wash Cart Reagent Block	15	N
18	146	Wash Large Loop (Cart)	10	N
19	107	Wash Output Block (S2)	15	N
20	137	Flush Input Block	10	N
21	136	Flush Cart Solvent Block	10	N
22	111	Wash Input Block (S3)	10	N
23	64	Del S3, Waste	10	N
24	135	Flush Cart Reagent Block	30	N
25	137	Flush Input Block	30	N
26	140	Flush Large Loop (Cart)	30	N
27	136	Flush Cart Solvent Block	60	N
28	138	Flush Output Block	30	N
29	131	Dry Cart (top)	40	N
30	63	Del S3, Cart (sensor)	20	N
31	61	Del S3, Cart (top)	10	N
32	148	Cartridge Wait	5	N
33	131	Dry Cart (top)	30	N
34	53	Del S2, Cart (sensor)	20	N
35	51	Del S2, Cart (top)	5	N
36	148	Cartridge Wait	5	N
37	51	Del S2, Cart (top)	5	N

Step	Function #	Function Name	Time (sec)	Global Time
38	148	Cartridge Wait	5	N
39	51	Del S2, Cart (top)	5	N
40	148	Cartridge Wait	5	N
41	61	Del S3, Cart (top)	5	N
42	148	Cartridge Wait	5	N
43	61	Del S3, Cart (top)	5	N
44	148	Cartridge Wait	5	N
45	61	Del S3, Cart (top)	5	N
46	131	Dry Cart (top)	120	N
47	137	Flush Input Block	5	N
48	11	Del R2g, Cart (top)	30	N
49	140	Flush Large Loop (Cart)	10	N
50	6	Load R1, Cart (lg loop)	20	N
51	131	Dry Cart (top)	30	N
52	140	Flush Large Loop (Cart)	5	N
53	135	Flush Cart Reagent Block	5	N
54	11	Del R2g, Cart (top)	170	N
55	132	Dry Cart (bottom)	30	N
56	140	Flush Large Loop (Cart)	5	N
57	6	Load R1, Cart (lg loop)	20	N
58	131	Dry Cart (top)	30	N
59	140	Flush Large Loop (Cart)	5	N
60	135	Flush Cart Reagent Block	5	N
61	11	Del R2g, Cart (top)	170	N
62	146	Wash Large Loop (Cart)	10	N
63	140	Flush Large Loop (Cart)	20	N
64	143	Wash Cart Reagent Block	15	N
65	135	Flush Cart Reagent Block	30	N
66	136	Flush Cart Solvent Block	30	N
67	132	Dry Cart (bottom)	60	N
68	63	Del S3, Cart (sensor)	20	N
69	131	Dry Cart (top)	30	N
70	63	Del S3, Cart (sensor)	20	N
71	131	Dry Cart (top)	30	N
72	53	Del S2, Cart (sensor)	20	N
73	51	Del S2, Cart (top)	5	N
74	148	Cartridge Wait	5	N
75	51	Del S2, Cart (top)	5	N
76	131	Dry Cart (top)	30	N
77	53	Del S2, Cart (sensor)	20	N
78	51	Del S2, Cart (top)	5	N

Step	Function #	Function Name	Time (sec)	Global Time
79	148	Cartridge Wait	5	N
80	51	Del S2, Cart (top)	5	N
81	131	Dry Cart (top)	30	N
82	63	Del S3, Cart (sensor)	20	N
83	131	Dry Cart (top)	30	N
84	63	Del S3, Cart (sensor)	20	N
85	131	Dry Cart (top)	30	N
86	63	Del S3, Cart (sensor)	20	N
87	131	Dry Cart (top)	60	N
88	259	End	0	N

Cart Begin Gas-phase

Total run time: 36:55

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	144	Wash Cart Solvent Block	10	N
3	136	Flush Cart Solvent Block	40	N
4	106	Wash Input Block (S2)	10	N
5	111	Wash Input Block (S3)	10	N
6	137	Flush Input Block	30	N
7	131	Dry Cart (top)	60	N
8	34	Del R3g, Waste	30	N
9	31	Del R3g, Cart (top)	450	N
10	143	Wash Cart Reagent Block	15	N
11	135	Flush Cart Reagent Block	40	N
12	107	Wash Output Block (S2)	15	N
13	144	Wash Cart Solvent Block	15	N
14	136	Flush Cart Solvent Block	40	N
15	138	Flush Output Block	40	N
16	131	Dry Cart (top)	40	N
17	63	Del S3, Cart (sensor)	20	N
18	61	Del S3, Cart (top)	10	N
19	148	Cartridge Wait	5	N
20	131	Dry Cart (top)	30	N
21	53	Del S2, Cart (sensor)	20	N
22	51	Del S2, Cart (top)	5	N
23	148	Cartridge Wait	5	N
24	51	Del S2, Cart (top)	5	N
25	148	Cartridge Wait	5	N
26	51	Del S2, Cart (top)	5	N

Step	Function #	Function Name	Time (sec)	Global Time
27	148	Cartridge Wait	5	N
28	61	Del S3, Cart (top)	5	N
29	148	Cartridge Wait	5	N
30	61	Del S3, Cart (top)	5	N
31	148	Cartridge Wait	5	N
32	61	Del S3, Cart (top)	5	N
33	148	Cartridge Wait	5	N
34	131	Dry Cart (top)	120	N
35	146	Wash Large Loop (Cart)	10	N
36	140	Flush Large Loop (Cart)	20	N
37	11	Del R2g, Cart (top)	30	N
38	140	Flush Large Loop (Cart)	5	N
39	6	Load R1, Cart (lg loop)	20	N
40	131	Dry Cart (top)	30	N
41	140	Flush Large Loop (Cart)	5	N
42	135	Flush Cart Reagent Block	5	N
43	11	Del R2g, Cart (top)	170	N
44	132	Dry Cart (bottom)	30	N
45	140	Flush Large Loop (Cart)	5	N
46	6	Load R1, Cart (lg loop)	20	N
47	140	Flush Large Loop (Cart)	5	N
48	135	Flush Cart Reagent Block	5	N
49	11	Del R2g, Cart (top)	170	N
50	146	Wash Large Loop (Cart)	10	N
51	140	Flush Large Loop (Cart)	20	N
52	143	Wash Cart Reagent Block	15	N
53	135	Flush Cart Reagent Block	30	N
54	136	Flush Cart Solvent Block	30	N
55	132	Dry Cart (bottom)	60	N
56	63	Del S3, Cart (sensor)	20	N
57	131	Dry Cart (top)	30	N
58	63	Del S3, Cart (sensor)	20	N
59	131	Dry Cart (top)	30	N
60	53	Del S2, Cart (sensor)	20	N
61	51	Del S2, Cart (top)	5	N
62	148	Cartridge Wait	5	N
63	131	Dry Cart (top)	30	N
64	53	Del S2, Cart (sensor)	20	N
65	51	Del S2, Cart (top)	5	N
66	148	Cartridge Wait	5	N
67	51	Del S2, Cart (top)	5	N

Step	Function #	Function Name	Time (sec)	Global Time
68	148	Cartridge Wait	5	N
69	131	Dry Cart (top)	30	N
70	63	Del S3, Cart (sensor)	20	N
71	131	Dry Cart (top)	30	N
72	63	Del S3, Cart (sensor)	20	N
73	131	Dry Cart (top)	30	N
74	63	Del S3, Cart (sensor)	20	N
75	131	Dry Cart (top)	60	N
76	259	End	0	N

Cart Precycle Total run time: 34:20

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	143	Wash Cart Reagent Block	10	N
3	135	Flush Cart Reagent Block	30	N
4	11	Del R2g, Cart (top)	60	N
5	140	Flush Large Loop (Cart)	10	N
6	6	Load R1, Cart (lg loop)	20	N
7	131	Dry Cart (top)	30	N
8	140	Flush Large Loop (Cart)	5	N
9	135	Flush Cart Reagent Block	5	N
10	11	Del R2g, Cart (top)	170	N
11	132	Dry Cart (bottom)	30	N
12	140	Flush Large Loop (Cart)	5	N
13	6	Load R1, Cart (lg loop)	20	N
14	131	Dry Cart (top)	30	N
15	140	Flush Large Loop (Cart)	5	N
16	135	Flush Cart Reagent Block	5	N
17	11	Del R2g, Cart (top)	170	N
18	146	Wash Large Loop (Cart)	10	N
19	140	Flush Large Loop (Cart)	20	N
20	143	Wash Cart Reagent Block	15	N
21	135	Flush Cart Reagent Block	30	N
22	136	Flush Cart Solvent Block	30	N
23	132	Dry Cart (bottom)	60	N
24	63	Del S3, Cart (sensor)	20	N
25	61	Del S3, Cart (top)	10	N
26	148	Cartridge Wait	5	N
27	51	Del S2, Cart (top)	10	N

Step	Function #	Function Name	Time (sec)	Global Time
28	148	Cartridge Wait	5	N
29	131	Dry Cart (top)	30	N
30	53	Del S2, Cart (sensor)	20	N
31	51	Del S2, Cart (top)	10	N
32	148	Cartridge Wait	5	N
33	61	Del S3, Cart (top)	10	N
34	148	Cartridge Wait	5	N
35	131	Dry Cart (top)	60	N
36	111	Wash Input Block (S3)	10	N
37	137	Flush Input Block	30	N
38	140	Flush Large Loop (Cart)	10	N
39	26	Load R3, Cart (lg loop)	50	N
40	30	Transfer R3, Cart (gas)	5	N
41	136	Flush Cart Solvent Block	15	N
42	131	Dry Cart (top)	30	N
43	53	Del S2, Cart (sensor)	20	N
44	51	Del S2, Cart (top)	10	N
45	148	Cartridge Wait	5	N
46	61	Del S3, Cart (top)	10	N
47	148	Cartridge Wait	5	N
48	131	Dry Cart (top)	30	N
49	140	Flush Large Loop (Cart)	10	N
50	26	Load R3, Cart (lg loop)	50	N
51	30	Transfer R3, Cart (gas)	5	N
52	136	Flush Cart Solvent Block	15	N
53	131	Dry Cart (top)	30	N
54	53	Del S2, Cart (sensor)	20	N
55	51	Del S2, Cart (top)	10	N
56	148	Cartridge Wait	5	N
57	61	Del S3, Cart (top)	10	N
58	148	Cartridge Wait	5	N
59	131	Dry Cart (top)	30	N
60	140	Flush Large Loop (Cart)	10	N
61	26	Load R3, Cart (lg loop)	50	N
62	30	Transfer R3, Cart (gas)	5	N
63	136	Flush Cart Solvent Block	15	N
64	131	Dry Cart (top)	30	N
65	53	Del S2, Cart (sensor)	20	N
66	51	Del S2, Cart (top)	10	N
67	148	Cartridge Wait	5	N
68	61	Del S3, Cart (top)	10	N

Step	Function #	Function Name	Time (sec)	Global Time
69	148	Cartridge Wait	5	N
70	131	Dry Cart (top)	30	N
71	140	Flush Large Loop (Cart)	10	N
72	26	Load R3, Cart (lg loop)	50	N
73	30	Transfer R3, Cart (gas)	5	N
74	136	Flush Cart Solvent Block	15	N
75	144	Wash Cart Solvent Block	10	N
76	146	Wash Large Loop (Cart)	10	N
77	140	Flush Large Loop (Cart)	30	N
78	136	Flush Cart Solvent Block	30	N
79	131	Dry Cart (top)	30	N
80	53	Del S2, Cart (sensor)	20	N
81	51	Del S2, Cart (top)	15	N
82	148	Cartridge Wait	5	N
83	51	Del S2, Cart (top)	15	N
84	148	Cartridge Wait	5	N
85	51	Del S2, Cart (top)	15	N
86	148	Cartridge Wait	5	N
87	131	Dry Cart (top)	30	N
88	63	Del S3, Cart (sensor)	20	N
89	131	Dry Cart (top)	30	N
90	63	Del S3, Cart (sensor)	20	N
91	131	Dry Cart (top)	120	N
92	259	End	0	N

Cart Pulsed-liquid Total run time: 33:45

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	137	Flush Input Block	5	N
3	11	Del R2g, Cart (top)	30	N
4	140	Flush Large Loop (Cart)	10	N
5	6	Load R1, Cart (lg loop)	20	N
6	131	Dry Cart (top)	30	N
7	140	Flush Large Loop (Cart)	5	N
8	135	Flush Cart Reagent Block	5	N
9	11	Del R2g, Cart (top)	170	N
10	132	Dry Cart (bottom)	30	N
11	140	Flush Large Loop (Cart)	5	N
12	6	Load R1, Cart (lg loop)	20	N

Step	Function #	Function Name	Time (sec)	Global Time
13	131	Dry Cart (top)	30	N
14	140	Flush Large Loop (Cart)	5	N
15	135	Flush Cart Reagent Block	5	N
16	11	Del R2g, Cart (top)	170	N
17	132	Dry Cart (bottom)	30	N
18	140	Flush Large Loop (Cart)	5	N
19	6	Load R1, Cart (lg loop)	20	N
20	131	Dry Cart (top)	30	N
21	140	Flush Large Loop (Cart)	5	N
22	135	Flush Cart Reagent Block	5	N
23	11	Del R2g, Cart (top)	170	N
24	146	Wash Large Loop (Cart)	10	N
25	111	Wash Input Block (S3)	10	N
26	140	Flush Large Loop (Cart)	15	N
27	137	Flush Input Block	20	N
28	136	Flush Cart Solvent Block	30	N
29	132	Dry Cart (bottom)	60	N
30	142	Set Cart Temperature	48	N
31	63	Del S3, Cart (sensor)	15	N
32	148	Cartridge Wait	10	N
33	61	Del S3, Cart (top)	10	N
34	148	Cartridge Wait	10	N
35	51	Del S2, Cart (top)	5	N
36	148	Cartridge Wait	5	N
37	51	Del S2, Cart (top)	5	N
38	148	Cartridge Wait	5	N
39	131	Dry Cart (top)	30	N
40	53	Del S2, Cart (sensor)	15	N
41	148	Cartridge Wait	5	N
42	51	Del S2, Cart (top)	5	N
43	148	Cartridge Wait	5	N
44	51	Del S2, Cart (top)	5	N
45	148	Cartridge Wait	5	N
46	61	Del S3, Cart (top)	10	N
47	148	Cartridge Wait	10	N
48	131	Dry Cart (top)	60	N
49	137	Flush Input Block	30	N
50	140	Flush Large Loop (Cart)	10	N
51	26	Load R3, Cart (lg loop)	50	N
52	30	Transfer R3, Cart (gas)	5	N
53	136	Flush Cart Solvent Block	10	N

Step	Function #	Function Name	Time (sec)	Global Time
54	140	Flush Large Loop (Cart)	10	N
55	138	Flush Output Block	10	N
56	144	Wash Cart Solvent Block	10	N
57	143	Wash Cart Reagent Block	15	N
58	146	Wash Large Loop (Cart)	10	N
59	107	Wash Output Block (S2)	15	N
60	137	Flush Input Block	10	N
61	136	Flush Cart Solvent Block	10	N
62	111	Wash Input Block (S3)	5	N
63	64	Del S3, Waste	5	N
64	135	Flush Cart Reagent Block	40	N
65	137	Flush Input Block	30	N
66	140	Flush Large Loop (Cart)	30	N
67	136	Flush Cart Solvent Block	60	N
68	138	Flush Output Block	30	N
69	257	Wait	0	Y
70	131	Dry Cart (top)	40	N
71	142	Set Cart Temperature	45	N
72	127	Ready Transfer to Flask	0	N
73	141	Flush Transfer Line	5	N
74	63	Del S3, Cart (sensor)	15	N
75	148	Cartridge Wait	10	N
76	121	Transfer to Flask (gas)	45	N
77	141	Flush Transfer Line	5	N
78	53	Del S2, Cart (sensor)	15	N
79	148	Cartridge Wait	10	N
80	121	Transfer to Flask (gas)	45	N
81	141	Flush Transfer Line	5	N
82	63	Del S3, Cart (sensor)	15	N
83	148	Cartridge Wait	10	N
84	121	Transfer to Flask (gas)	45	N
85	128	Transfer Complete	0	N
86	131	Dry Cart (top)	60	N
87	61	Del S3, Cart (top)	15	N
88	148	Cartridge Wait	5	N
89	131	Dry Cart (top)	120	N
90	259	End	0	N

Cart Gas-phase Total run time: 41:05

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	137	Flush Input Block	5	N
3	11	Del R2g, Cart (top)	30	N
4	140	Flush Large Loop (Cart)	10	N
5	6	Load R1, Cart (lg loop)	20	N
6	131	Dry Cart (top)	30	N
7	140	Flush Large Loop (Cart)	5	N
8	135	Flush Cart Reagent Block	5	N
9	11	Del R2g, Cart (top)	170	N
10	132	Dry Cart (bottom)	30	N
11	140	Flush Large Loop (Cart)	5	N
12	6	Load R1, Cart (lg loop)	20	N
13	131	Dry Cart (top)	30	N
14	140	Flush Large Loop (Cart)	5	N
15	135	Flush Cart Reagent Block	5	N
16	11	Del R2g, Cart (top)	170	N
17	132	Dry Cart (bottom)	30	N
18	140	Flush Large Loop (Cart)	5	N
19	6	Load R1, Cart (lg loop)	20	N
20	131	Dry Cart (top)	30	N
21	140	Flush Large Loop (Cart)	5	N
22	135	Flush Cart Reagent Block	5	N
23	11	Del R2g, Cart (top)	170	N
24	146	Wash Large Loop (Cart)	10	N
25	111	Wash Input Block (S3)	10	N
26	140	Flush Large Loop (Cart)	15	N
27	137	Flush Input Block	20	N
28	136	Flush Cart Solvent Block	30	N
29	132	Dry Cart (bottom)	60	N
30	142	Set Cart Temperature	48	N
31	63	Del S3, Cart (sensor)	15	N
32	148	Cartridge Wait	10	N
33	61	Del S3, Cart (top)	10	N
34	148	Cartridge Wait	10	N
35	51	Del S2, Cart (top)	5	N
36	148	Cartridge Wait	5	N
37	51	Del S2, Cart (top)	5	N
38	148	Cartridge Wait	5	N
39	131	Dry Cart (top)	30	N

Step	Function #	Function Name	Time (sec)	Global Time
40	53	Del S2, Cart (sensor)	15	N
41	148	Cartridge Wait	5	N
42	51	Del S2, Cart (top)	5	N
43	148	Cartridge Wait	5	N
44	51	Del S2, Cart (top)	5	N
45	148	Cartridge Wait	5	N
46	61	Del S3, Cart (top)	10	N
47	148	Cartridge Wait	10	N
48	131	Dry Cart (top)	60	N
49	34	Del R3g, Waste	30	N
50	31	Del R3g, Cart (top)	720	N
51	143	Wash Cart Reagent Block	15	N
52	135	Flush Cart Reagent Block	30	N
53	144	Wash Cart Solvent Block	10	N
54	136	Flush Cart Solvent Block	30	N
55	131	Dry Cart (top)	40	N
56	142	Set Cart Temperature	45	N
57	127	Ready Transfer to Flask	0	N
58	141	Flush Transfer Line	5	N
59	63	Del S3, Cart (sensor)	15	N
60	148	Cartridge Wait	10	N
61	121	Transfer to Flask (gas)	45	N
62	141	Flush Transfer Line	5	N
63	53	Del S2, Cart (sensor)	15	N
64	148	Cartridge Wait	10	N
65	121	Transfer to Flask (gas)	45	N
66	141	Flush Transfer Line	5	N
67	63	Del S3, Cart (sensor)	15	N
68	148	Cartridge Wait	10	N
69	121	Transfer to Flask (gas)	45	N
70	128	Transfer Complete	0	N
71	131	Dry Cart (top)	60	N
72	61	Del S3, Cart (top)	15	N
73	148	Cartridge Wait	5	N
74	131	Dry Cart (top)	120	N
75	259	End	0	N

Cart PL PVDF Protein

Total run time: 35:45

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	139	Flush Small Loop (Cart)	10	N
3	75	Load X1, Cart (sm loop)	70	Y
4	131	Dry Cart (top)	30	N
5	139	Flush Small Loop (Cart)	10	N
6	135	Flush Cart Reagent Block	5	N
7	11	Del R2g, Cart (top)	30	N
8	140	Flush Large Loop (Cart)	10	N
9	6	Load R1, Cart (lg loop)	20	N
10	131	Dry Cart (top)	30	N
11	140	Flush Large Loop (Cart)	5	N
12	135	Flush Cart Reagent Block	5	N
13	11	Del R2g, Cart (top)	170	N
14	132	Dry Cart (bottom)	30	N
15	140	Flush Large Loop (Cart)	5	N
16	6	Load R1, Cart (lg loop)	20	N
17	131	Dry Cart (top)	30	N
18	140	Flush Large Loop (Cart)	5	N
19	135	Flush Cart Reagent Block	5	N
20	11	Del R2g, Cart (top)	170	N
21	132	Dry Cart (bottom)	30	N
22	140	Flush Large Loop (Cart)	5	N
23	6	Load R1, Cart (lg loop)	20	N
24	131	Dry Cart (top)	30	N
25	140	Flush Large Loop (Cart)	5	N
26	135	Flush Cart Reagent Block	5	N
27	11	Del R2g, Cart (top)	170	N
28	146	Wash Large Loop (Cart)	10	N
29	111	Wash Input Block (S3)	10	N
30	140	Flush Large Loop (Cart)	15	N
31	137	Flush Input Block	20	N
32	136	Flush Cart Solvent Block	30	N
33	132	Dry Cart (bottom)	60	N
34	142	Set Cart Temperature	48	N
35	63	Del S3, Cart (sensor)	15	N
36	148	Cartridge Wait	10	N
37	61	Del S3, Cart (top)	10	N
38	148	Cartridge Wait	10	N
39	51	Del S2, Cart (top)	5	N

Step	Function #	Function Name	Time (sec)	Global Time
40	148	Cartridge Wait	5	N
41	51	Del S2, Cart (top)	5	N
42	148	Cartridge Wait	5	N
43	131	Dry Cart (top)	30	N
44	53	Del S2, Cart (sensor)	15	N
45	148	Cartridge Wait	5	N
46	51	Del S2, Cart (top)	5	N
47	148	Cartridge Wait	5	N
48	51	Del S2, Cart (top)	5	N
49	148	Cartridge Wait	5	N
50	61	Del S3, Cart (top)	10	N
51	148	Cartridge Wait	10	N
52	131	Dry Cart (top)	60	N
53	137	Flush Input Block	30	N
54	140	Flush Large Loop (Cart)	10	N
55	26	Load R3, Cart (lg loop)	50	N
56	30	Transfer R3, Cart (gas)	5	N
57	136	Flush Cart Solvent Block	10	N
58	140	Flush Large Loop (Cart)	10	N
59	138	Flush Output Block	10	N
60	144	Wash Cart Solvent Block	10	N
61	143	Wash Cart Reagent Block	15	N
62	146	Wash Large Loop (Cart)	10	N
63	107	Wash Output Block (S2)	15	N
64	137	Flush Input Block	10	N
65	136	Flush Cart Solvent Block	10	N
66	111	Wash Input Block (S3)	5	N
67	64	Del S3, Waste	5	N
68	135	Flush Cart Reagent Block	40	N
69	137	Flush Input Block	30	N
70	140	Flush Large Loop (Cart)	30	N
71	136	Flush Cart Solvent Block	60	N
72	138	Flush Output Block	30	N
73	257	Wait	0	Y
74	131	Dry Cart (top)	40	N
75	142	Set Cart Temperature	48	N
76	127	Ready Transfer to Flask	0	N
77	141	Flush Transfer Line	5	N
78	63	Del S3, Cart (sensor)	15	N
79	148	Cartridge Wait	10	N
80	121	Transfer to Flask (gas)	45	N

Step	Function #	Function Name	Time (sec)	Global Time
81	141	Flush Transfer Line	5	N
82	53	Del S2, Cart (sensor)	15	N
83	148	Cartridge Wait	10	N
84	121	Transfer to Flask (gas)	45	N
85	141	Flush Transfer Line	5	N
86	63	Del S3, Cart (sensor)	15	N
87	148	Cartridge Wait	10	N
88	121	Transfer to Flask (gas)	45	N
89	128	Transfer Complete	0	N
90	131	Dry Cart (top)	60	N
91	61	Del S3, Cart (top)	15	N
92	148	Cartridge Wait	5	N
93	131	Dry Cart (top)	120	N
94	259	End	0	N

Cart GP PVDF Protein

Total run time: 43:05

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	139	Flush Small Loop (Cart)	10	N
3	75	Load X1, Cart (sm loop)	70	Y
4	131	Dry Cart (top)	30	N
5	139	Flush Small Loop (Cart)	10	N
6	135	Flush Cart Reagent Block	5	N
7	11	Del R2g, Cart (top)	30	N
8	140	Flush Large Loop (Cart)	10	N
9	6	Load R1, Cart (lg loop)	20	N
10	131	Dry Cart (top)	30	N
11	140	Flush Large Loop (Cart)	5	N
12	135	Flush Cart Reagent Block	5	N
13	11	Del R2g, Cart (top)	170	N
14	132	Dry Cart (bottom)	30	N
15	140	Flush Large Loop (Cart)	5	N
16	6	Load R1, Cart (lg loop)	20	N
17	131	Dry Cart (top)	30	N
18	140	Flush Large Loop (Cart)	5	N
19	135	Flush Cart Reagent Block	5	N
20	11	Del R2g, Cart (top)	170	N
21	132	Dry Cart (bottom)	30	N
22	140	Flush Large Loop (Cart)	5	N

Step	Function #	Function Name	Time (sec)	Global Time
23	6	Load R1, Cart (lg loop)	20	N
24	131	Dry Cart (top)	30	N
25	140	Flush Large Loop (Cart)	5	N
26	135	Flush Cart Reagent Block	5	N
27	11	Del R2g, Cart (top)	170	N
28	146	Wash Large Loop (Cart)	10	N
29	111	Wash Input Block (S3)	10	N
30	140	Flush Large Loop (Cart)	15	N
31	137	Flush Input Block	20	N
32	136	Flush Cart Solvent Block	30	N
33	132	Dry Cart (bottom)	60	N
34	142	Set Cart Temperature	48	N
35	63	Del S3, Cart (sensor)	15	N
36	148	Cartridge Wait	10	N
37	61	Del S3, Cart (top)	10	N
38	148	Cartridge Wait	10	N
39	51	Del S2, Cart (top)	5	N
40	148	Cartridge Wait	5	N
41	51	Del S2, Cart (top)	5	N
42	148	Cartridge Wait	5	N
43	131	Dry Cart (top)	30	N
44	53	Del S2, Cart (sensor)	15	N
45	148	Cartridge Wait	5	N
46	51	Del S2, Cart (top)	5	N
47	148	Cartridge Wait	5	N
48	51	Del S2, Cart (top)	5	N
49	148	Cartridge Wait	5	N
50	61	Del S3, Cart (top)	10	N
51	148	Cartridge Wait	10	N
52	131	Dry Cart (top)	60	N
53	34	Del R3g, Waste	30	N
54	31	Del R3g, Cart (top)	720	N
55	143	Wash Cart Reagent Block	15	N
56	135	Flush Cart Reagent Block	30	N
57	144	Wash Cart Solvent Block	10	N
58	136	Flush Cart Solvent Block	30	N
59	131	Dry Cart (top)	40	N
60	142	Set Cart Temperature	48	N
61	127	Ready Transfer to Flask	0	N
62	141	Flush Transfer Line	5	N
63	63	Del S3, Cart (sensor)	15	N

Step	Function #	Function Name	Time (sec)	Global Time
64	148	Cartridge Wait	10	N
65	121	Transfer to Flask (gas)	45	N
66	141	Flush Transfer Line	5	N
67	53	Del S2, Cart (sensor)	15	N
68	148	Cartridge Wait	10	N
69	121	Transfer to Flask (gas)	45	N
70	141	Flush Transfer Line	5	N
71	63	Del S3, Cart (sensor)	15	N
72	148	Cartridge Wait	10	N
73	121	Transfer to Flask (gas)	45	N
74	128	Transfer Complete	0	N
75	131	Dry Cart (top)	60	N
76	61	Del S3, Cart (top)	15	N
77	148	Cartridge Wait	5	N
78	131	Dry Cart (top)	120	N
79	259	End	0	N

Cart PL PVDF Peptide

Total run time: 33:45

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	137	Flush Input Block	5	N
3	11	Del R2g, Cart (top)	30	N
4	140	Flush Large Loop (Cart)	10	N
5	6	Load R1, Cart (lg loop)	20	N
6	131	Dry Cart (top)	30	N
7	140	Flush Large Loop (Cart)	5	N
8	135	Flush Cart Reagent Block	5	N
9	11	Del R2g, Cart (top)	170	N
10	132	Dry Cart (bottom)	30	N
11	140	Flush Large Loop (Cart)	5	N
12	6	Load R1, Cart (lg loop)	20	N
13	131	Dry Cart (top)	30	N
14	140	Flush Large Loop (Cart)	5	N
15	135	Flush Cart Reagent Block	5	N
16	11	Del R2g, Cart (top)	170	N
17	132	Dry Cart (bottom)	30	N
18	140	Flush Large Loop (Cart)	5	N
19	6	Load R1, Cart (lg loop)	20	N
20	131	Dry Cart (top)	30	N

Step	Function #	Function Name	Time (sec)	Global Time
21	140	Flush Large Loop (Cart)	5	N
22	135	Flush Cart Reagent Block	5	N
23	11	Del R2g, Cart (top)	170	N
24	146	Wash Large Loop (Cart)	10	N
25	111	Wash Input Block (S3)	10	N
26	140	Flush Large Loop (Cart)	15	N
27	137	Flush Input Block	20	N
28	136	Flush Cart Solvent Block	30	N
29	132	Dry Cart (bottom)	60	N
30	142	Set Cart Temperature	48	N
31	63	Del S3, Cart (sensor)	15	N
32	148	Cartridge Wait	10	N
33	61	Del S3, Cart (top)	10	N
34	148	Cartridge Wait	10	N
35	51	Del S2, Cart (top)	5	N
36	148	Cartridge Wait	5	N
37	51	Del S2, Cart (top)	5	N
38	148	Cartridge Wait	5	N
39	131	Dry Cart (top)	30	N
40	53	Del S2, Cart (sensor)	15	N
41	148	Cartridge Wait	5	N
42	51	Del S2, Cart (top)	5	N
43	148	Cartridge Wait	5	N
44	51	Del S2, Cart (top)	5	N
45	148	Cartridge Wait	5	N
46	61	Del S3, Cart (top)	10	N
47	148	Cartridge Wait	10	N
48	131	Dry Cart (top)	60	N
49	137	Flush Input Block	30	N
50	140	Flush Large Loop (Cart)	10	N
51	26	Load R3, Cart (lg loop)	50	N
52	30	Transfer R3, Cart (gas)	5	N
53	136	Flush Cart Solvent Block	10	N
54	140	Flush Large Loop (Cart)	10	N
55	138	Flush Output Block	10	N
56	144	Wash Cart Solvent Block	10	N
57	143	Wash Cart Reagent Block	15	N
58	146	Wash Large Loop (Cart)	10	N
59	107	Wash Output Block (S2)	15	N
60	137	Flush Input Block	10	N
61	136	Flush Cart Solvent Block	10	N

Step	Function #	Function Name	Time (sec)	Global Time
62	111	Wash Input Block (S3)	5	N
63	64	Del S3, Waste	5	N
64	135	Flush Cart Reagent Block	40	N
65	137	Flush Input Block	30	N
66	140	Flush Large Loop (Cart)	30	N
67	136	Flush Cart Solvent Block	60	N
68	138	Flush Output Block	30	N
69	257	Wait	0	Y
70	131	Dry Cart (top)	40	N
71	142	Set Cart Temperature	48	N
72	127	Ready Transfer to Flask	0	N
73	141	Flush Transfer Line	5	N
74	63	Del S3, Cart (sensor)	15	N
75	148	Cartridge Wait	10	N
76	121	Transfer to Flask (gas)	45	N
77	141	Flush Transfer Line	5	N
78	53	Del S2, Cart (sensor)	15	N
79	148	Cartridge Wait	10	N
80	121	Transfer to Flask (gas)	45	N
81	141	Flush Transfer Line	5	N
82	63	Del S3, Cart (sensor)	15	N
83	148	Cartridge Wait	10	N
84	121	Transfer to Flask (gas)	45	N
85	128	Transfer Complete	0	N
86	131	Dry Cart (top)	60	N
87	61	Del S3, Cart (top)	15	N
88	148	Cartridge Wait	5	N
89	131	Dry Cart (top)	120	N
90	259	End	0	N

Cart GP PVDF Peptide

Total run time: 41:05

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	137	Flush Input Block	5	N
3	11	Del R2g, Cart (top)	30	N
4	140	Flush Large Loop (Cart)	10	N
5	6	Load R1, Cart (lg loop)	20	N
6	131	Dry Cart (top)	30	N
7	140	Flush Large Loop (Cart)	5	N

Step	Function #	Function Name	Time (sec)	Global Time
8	135	Flush Cart Reagent Block	5	N
9	11	Del R2g, Cart (top)	170	N
10	132	Dry Cart (bottom)	30	N
11	140	Flush Large Loop (Cart)	5	N
12	6	Load R1, Cart (lg loop)	20	N
13	131	Dry Cart (top)	30	N
14	140	Flush Large Loop (Cart)	5	N
15	135	Flush Cart Reagent Block	5	N
16	11	Del R2g, Cart (top)	170	N
17	132	Dry Cart (bottom)	30	N
18	140	Flush Large Loop (Cart)	5	N
19	6	Load R1, Cart (lg loop)	20	N
20	131	Dry Cart (top)	30	N
21	140	Flush Large Loop (Cart)	5	N
22	135	Flush Cart Reagent Block	5	N
23	11	Del R2g, Cart (top)	170	N
24	146	Wash Large Loop (Cart)	10	N
25	111	Wash Input Block (S3)	10	N
26	140	Flush Large Loop (Cart)	15	N
27	137	Flush Input Block	20	N
28	136	Flush Cart Solvent Block	30	N
29	132	Dry Cart (bottom)	60	N
30	142	Set Cart Temperature	48	N
31	63	Del S3, Cart (sensor)	15	N
32	148	Cartridge Wait	10	N
33	61	Del S3, Cart (top)	10	N
34	148	Cartridge Wait	10	N
35	51	Del S2, Cart (top)	5	N
36	148	Cartridge Wait	5	N
37	51	Del S2, Cart (top)	5	N
38	148	Cartridge Wait	5	N
39	131	Dry Cart (top)	30	N
40	53	Del S2, Cart (sensor)	15	N
41	148	Cartridge Wait	5	N
42	51	Del S2, Cart (top)	5	N
43	148	Cartridge Wait	5	N
44	51	Del S2, Cart (top)	5	N
45	148	Cartridge Wait	5	N
46	61	Del S3, Cart (top)	10	N
47	148	Cartridge Wait	10	N
48	131	Dry Cart (top)	60	N

Step	Function #	Function Name	Time (sec)	Global Time
49	34	Del R3g, Waste	30	N
50	31	Del R3g, Cart (top)	720	N
51	143	Wash Cart Reagent Block	15	N
52	135	Flush Cart Reagent Block	30	N
53	144	Wash Cart Solvent Block	10	N
54	136	Flush Cart Solvent Block	30	N
55	131	Dry Cart (top)	40	N
56	142	Set Cart Temperature	48	N
57	127	Ready Transfer to Flask	0	N
58	141	Flush Transfer Line	5	N
59	63	Del S3, Cart (sensor)	15	N
60	148	Cartridge Wait	10	N
61	121	Transfer to Flask (gas)	45	N
62	141	Flush Transfer Line	5	N
63	53	Del S2, Cart (sensor)	15	N
64	148	Cartridge Wait	10	N
65	121	Transfer to Flask (gas)	45	N
66	141	Flush Transfer Line	5	N
67	63	Del S3, Cart (sensor)	15	N
68	148	Cartridge Wait	10	N
69	121	Transfer to Flask (gas)	45	N
70	128	Transfer Complete	0	N
71	131	Dry Cart (top)	60	N
72	61	Del S3, Cart (top)	15	N
73	148	Cartridge Wait	5	N
74	131	Dry Cart (top)	120	N
75	259	End	0	N

Flask Optimize-Cart

Total run time: 5:25

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	131	Dry Cart (top)	40	N
3	127	Ready Transfer to Flask	0	N
4	141	Flush Transfer Line	5	N
5	63	Del S3, Cart (sensor)	15	N
6	148	Cartridge Wait	10	N
7	121	Transfer to Flask (gas)	45	N
8	141	Flush Transfer Line	5	N
9	53	Del S2, Cart (sensor)	15	N

Step	Function #	Function Name	Time (sec)	Global Time
10	148	Cartridge Wait	10	N
11	121	Transfer to Flask (gas)	45	N
12	141	Flush Transfer Line	5	N
13	63	Del S3, Cart (sensor)	15	N
14	148	Cartridge Wait	10	N
15	121	Transfer to Flask (gas)	45	N
16	128	Transfer Complete	0	N
17	131	Dry Cart (top)	60	N
18	259	End	0	N

Cart PL Proline Total run time: 33:45

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	137	Flush Input Block	5	N
3	11	Del R2g, Cart (top)	30	N
4	140	Flush Large Loop (Cart)	10	N
5	6	Load R1, Cart (lg loop)	20	N
6	131	Dry Cart (top)	30	N
7	140	Flush Large Loop (Cart)	5	N
8	135	Flush Cart Reagent Block	5	N
9	11	Del R2g, Cart (top)	170	N
10	132	Dry Cart (bottom)	30	N
11	140	Flush Large Loop (Cart)	5	N
12	6	Load R1, Cart (lg loop)	20	N
13	131	Dry Cart (top)	30	N
14	140	Flush Large Loop (Cart)	5	N
15	135	Flush Cart Reagent Block	5	N
16	11	Del R2g, Cart (top)	170	N
17	132	Dry Cart (bottom)	30	N
18	140	Flush Large Loop (Cart)	5	N
19	6	Load R1, Cart (lg loop)	20	N
20	131	Dry Cart (top)	30	N
21	140	Flush Large Loop (Cart)	5	N
22	135	Flush Cart Reagent Block	5	N
23	11	Del R2g, Cart (top)	170	N
24	146	Wash Large Loop (Cart)	10	N
25	111	Wash Input Block (S3)	10	N
26	140	Flush Large Loop (Cart)	15	N
27	137	Flush Input Block	20	N

Step	Function #	Function Name	Time (sec)	Global Time
28	136	Flush Cart Solvent Block	30	N
29	132	Dry Cart (bottom)	60	N
30	142	Set Cart Temperature	53	N
31	63	Del S3, Cart (sensor)	15	N
32	148	Cartridge Wait	10	N
33	61	Del S3, Cart (top)	10	N
34	148	Cartridge Wait	10	N
35	51	Del S2, Cart (top)	5	N
36	148	Cartridge Wait	5	N
37	51	Del S2, Cart (top)	5	N
38	148	Cartridge Wait	5	N
39	131	Dry Cart (top)	30	N
40	53	Del S2, Cart (sensor)	15	N
41	148	Cartridge Wait	5	N
42	51	Del S2, Cart (top)	5	N
43	148	Cartridge Wait	5	N
44	51	Del S2, Cart (top)	5	N
45	148	Cartridge Wait	5	N
46	61	Del S3, Cart (top)	10	N
47	148	Cartridge Wait	10	N
48	131	Dry Cart (top)	60	N
49	137	Flush Input Block	30	N
50	140	Flush Large Loop (Cart)	10	N
51	26	Load R3, Cart (lg loop)	50	N
52	30	Transfer R3, Cart (gas)	5	N
53	136	Flush Cart Solvent Block	10	N
54	140	Flush Large Loop (Cart)	10	N
55	138	Flush Output Block	10	N
56	144	Wash Cart Solvent Block	10	N
57	143	Wash Cart Reagent Block	15	N
58	146	Wash Large Loop (Cart)	10	N
59	107	Wash Output Block (S2)	15	N
60	137	Flush Input Block	10	N
61	136	Flush Cart Solvent Block	10	N
62	111	Wash Input Block (S3)	5	N
63	64	Del S3, Waste	5	N
64	135	Flush Cart Reagent Block	40	N
65	137	Flush Input Block	30	N
66	140	Flush Large Loop (Cart)	30	N
67	136	Flush Cart Solvent Block	60	N
68	138	Flush Output Block	30	N

Step	Function #	Function Name	Time (sec)	Global Time
69	257	Wait	0	Y
70	131	Dry Cart (top)	40	N
71	142	Set Cart Temperature	45	N
72	127	Ready Transfer to Flask	0	N
73	141	Flush Transfer Line	5	N
74	63	Del S3, Cart (sensor)	15	N
75	148	Cartridge Wait	10	N
76	121	Transfer to Flask (gas)	45	N
77	141	Flush Transfer Line	5	N
78	53	Del S2, Cart (sensor)	15	N
79	148	Cartridge Wait	10	N
80	121	Transfer to Flask (gas)	45	N
81	141	Flush Transfer Line	5	N
82	63	Del S3, Cart (sensor)	15	N
83	148	Cartridge Wait	10	N
84	121	Transfer to Flask (gas)	45	N
85	128	Transfer Complete	0	N
86	131	Dry Cart (top)	60	N
87	61	Del S3, Cart (top)	15	N
88	148	Cartridge Wait	5	N
89	131	Dry Cart (top)	120	N
90	259	End	0	N

Flask Cycle List

Flask Blank Total run time: 35:57

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	233	Set as Blank Cycle	0	N
3	171	Del S4, Flask	15	N
4	213	Dry Flask	10	N
5	215	Empty Flask	20	N
6	151	Del R4, Flask	15	N
7	213	Dry Flask	10	N
8	215	Empty Flask	20	N
9	218	Flush Large Loop (Flask)	10	N
10	173	Load S4, Flask (lg loop)	15	N
11	213	Dry Flask	10	N
12	218	Flush Large Loop (Flask)	10	N
13	257	Wait	30	N
14	213	Dry Flask	150	N
15	236	Pre-Conversion Dry	0	Y
16	218	Flush Large Loop (Flask)	10	N
17	153	Load R4, Flask (lg loop)	20	N
18	213	Dry Flask	10	N
19	218	Flush Large Loop (Flask)	10	N
20	173	Load S4, Flask (lg loop)	15	N
21	218	Flush Large Loop (Flask)	15	N
22	257	Wait	540	N
23	237	Post-Conversion Dry	0	Y
24	226	Load Position	1	N
25	227	Prepare Pump	1	N
26	213	Dry Flask	450	N
27	173	Load S4, Flask (lg loop)	15	N
28	213	Dry Flask	10	N
29	218	Flush Large Loop (Flask)	10	N
30	173	Load S4, Flask (lg loop)	15	N
31	213	Dry Flask	10	N
32	218	Flush Large Loop (Flask)	10	N
33	221	Flush Injector	30	N
34	257	Wait	5	N
35	221	Flush Injector	30	N
36	213	Dry Flask	5	N
37	238	Concentrate Sample	0	Y

Step	Function #	Function Name	Time (sec)	Global Time
38	257	Wait	10	N
39	225	Load Injector	40	N
40	249	Inject Pos/Collect Data	1	N
41	171	Del S4, Flask	15	N
42	213	Dry Flask	10	N
43	212	Bubble Flask	5	N
44	215	Empty Flask	10	N
45	171	Del S4, Flask	10	N
46	213	Dry Flask	10	N
47	212	Bubble Flask	5	N
48	222	Flush Flask/Injector	30	N
49	221	Flush Injector	10	N
50	259	End	0	N

Flask Standard Total run time: 36:07

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	234	Set as Standard Cycle	0	N
3	171	Del S4, Flask	15	N
4	213	Dry Flask	10	N
5	215	Empty Flask	20	N
6	151	Del R4, Flask	15	N
7	213	Dry Flask	10	N
8	215	Empty Flask	20	N
9	218	Flush Large Loop (Flask)	10	N
10	257	Wait	150	N
11	163	Load R5, Flask (lg loop)	20	N
12	213	Dry Flask	60	N
13	236	Pre-Conversion Dry	0	Y
14	218	Flush Large Loop (Flask)	10	N
15	153	Load R4, Flask (lg loop)	20	N
16	213	Dry Flask	10	N
17	218	Flush Large Loop (Flask)	10	N
18	173	Load S4, Flask (lg loop)	15	N
19	218	Flush Large Loop (Flask)	10	N
20	257	Wait	540	N
21	237	Post-Conversion Dry	0	Y
22	226	Load Position	1	N
23	227	Prepare Pump	1	N

Step	Function #	Function Name	Time (sec)	Global Time
24	213	Dry Flask	150	N
25	257	Wait	300	N
26	173	Load S4, Flask (lg loop)	15	N
27	213	Dry Flask	10	N
28	218	Flush Large Loop (Flask)	10	N
29	173	Load S4, Flask (lg loop)	15	N
30	213	Dry Flask	10	N
31	218	Flush Large Loop (Flask)	10	N
32	221	Flush Injector	30	N
33	257	Wait	5	N
34	221	Flush Injector	30	N
35	213	Dry Flask	5	N
36	238	Concentrate Sample	0	Y
37	257	Wait	10	N
38	225	Load Injector	40	N
39	249	Inject Pos/Collect Data	1	N
40	171	Del S4, Flask	15	N
41	213	Dry Flask	10	N
42	212	Bubble Flask	5	N
43	215	Empty Flask	10	N
44	171	Del S4, Flask	10	N
45	213	Dry Flask	10	N
46	212	Bubble Flask	5	N
47	222	Flush Flask/Injector	30	N
48	221	Flush Injector	10	N
49	259	End	0	N

Flask Normal Total run time: 31:38

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	235	Set as Residue Cycle	0	N
3	218	Flush Large Loop (Flask)	10	N
4	173	Load S4, Flask (lg loop)	15	N
5	213	Dry Flask	10	N
6	218	Flush Large Loop (Flask)	10	N
7	228	Ready to Receive	1	N
8	213	Dry Flask	15	N
9	236	Pre-Conversion Dry	0	Y
10	218	Flush Large Loop (Flask)	10	N

Step	Function #	Function Name	Time (sec)	Global Time
11	153	Load R4, Flask (lg loop)	20	N
12	213	Dry Flask	10	N
13	218	Flush Large Loop (Flask)	10	N
14	173	Load S4, Flask (lg loop)	15	N
15	218	Flush Large Loop (Flask)	10	N
16	257	Wait	540	N
17	237	Post-Conversion Dry	0	Y
18	226	Load Position	1	N
19	227	Prepare Pump	1	N
20	213	Dry Flask	450	N
21	173	Load S4, Flask (lg loop)	15	N
22	213	Dry Flask	10	N
23	218	Flush Large Loop (Flask)	10	N
24	173	Load S4, Flask (lg loop)	15	N
25	213	Dry Flask	10	N
26	218	Flush Large Loop (Flask)	10	N
27	221	Flush Injector	30	N
28	257	Wait	5	N
29	221	Flush Injector	30	N
30	213	Dry Flask	5	N
31	238	Concentrate Sample	0	Y
32	257	Wait	10	N
33	225	Load Injector	40	N
34	249	Inject Pos/Collect Data	1	N
35	171	Del S4, Flask	15	N
36	213	Dry Flask	10	N
37	212	Bubble Flask	5	N
38	215	Empty Flask	10	N
39	171	Del S4, Flask	10	N
40	213	Dry Flask	10	N
41	212	Bubble Flask	5	N
42	222	Flush Flask/Injector	30	N
43	221	Flush Injector	10	N
44	259	End	0	N

Run Gradient

Total run time: 31:32

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	257	Wait	30	N

Step	Function #	Function Name	Time (sec)	Global Time
3	227	Prepare Pump	1	N
4	257	Wait	480	N
5	232	Start Gradient	1	N
6	257	Wait	690	N
7	257	Wait	690	N
8	259	End	0	N

Flask Prep Pump

Total run time: 1:21

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	257	Wait	30	N
3	227	Prepare Pump	1	N
4	257	Wait	50	N
5	259	End	0	N

Injector Optimize

Total run time: 4:36

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	235	Set as Residue Cycle	0	N
3	218	Flush Large Loop (Flask)	10	N
4	215	Empty Flask	20	N
5	226	Load Position	1	N
6	173	Load S4, Flask (lg loop)	15	N
7	213	Dry Flask	10	N
8	218	Flush Large Loop (Flask)	10	N
9	173	Load S4, Flask (lg loop)	15	N
10	213	Dry Flask	10	N
11	218	Flush Large Loop (Flask)	10	N
12	221	Flush Injector	30	N
13	257	Wait	5	N
14	221	Flush Injector	30	N
15	213	Dry Flask	5	N
16	238	Concentrate Sample	0	Y
17	257	Wait	10	N
18	225	Load Injector	40	N
19	249	Inject Pos/Collect Data	1	N
20	222	Flush Flask/Injector	30	N
21	221	Flush Injector	10	N

Step	Function #	Function Name	Time (sec)	Global Time
22	259	End	0	N

Manual Injection Total run time: 31:33

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	226	Load Position	1	N
3	257	Wait	30	N
4	227	Prepare Pump	1	N
5	257	Wait	480	N
6	249	Inject Pos/Collect Data	1	N
7	232	Start Gradient	1	N
8	257	Wait	690	N
9	257	Wait	690	N
10	259	End	0	N

Flask Optimize-Flsk Total run time: 19:31

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	235	Set as Residue Cycle	0	N
3	218	Flush Large Loop (Flask)	10	N
4	215	Empty Flask	20	N
5	173	Load S4, Flask (lg loop)	15	N
6	213	Dry Flask	10	N
7	218	Flush Large Loop (Flask)	10	N
8	228	Ready to Receive	1	N
9	213	Dry Flask	120	N
10	301	Pause	0	N
11	236	Pre-Conversion Dry	0	Y
12	301	Pause	0	N
13	218	Flush Large Loop (Flask)	10	N
14	153	Load R4, Flask (lg loop)	20	N
15	213	Dry Flask	10	N
16	218	Flush Large Loop (Flask)	10	N
17	173	Load S4, Flask (lg loop)	15	N
18	218	Flush Large Loop (Flask)	10	N
19	301	Pause	0	N
20	213	Dry Flask	450	N
21	237	Post-Conversion Dry	0	Y

Step	Function #	Function Name	Time (sec)	Global Time
22	259	End	0	N

Method List

Methods Table

Method Name		Cart. Temp °C	Flask Temp °C	Column Temp °C
Filter Precycle		48	64	55
	Default	Cart Pulsed-liquid	Flask Normal	Fast Normal I
	1	None	Flask Prep Pump	Prep Pump
	2	Cart Precycle	Flask Blank	Fast Normal I
	3	Cart Precycle	Flask Standard	Fast Normal I
Fast Precycle		48	64	55
	Default	Cart Precycle	None	None
	1	Cart Precycle	Flask Standard	Fast Normal I
Pulsed-liquid		45	64	55
	Default	Cart Pulsed-liquid	Flask Normal	Fast Normal I
	1	None	Flask Prep Pump	Prep Pump
	2	None	Flask Blank	Fast Normal I
	3	Cart Begin	Flask Standard	Fast Normal I
Gas-phase		45	64	55
	Default	Cart Gas-phase	Flask Normal	Fast Normal I
	1	None	Flask Prep Pump	Prep Pump
	2	None	Flask Blank	Fast Normal I
	3	Cart Begin Gas-phase	Flask Standard	Fast Normal I
PL PVDF Protein		48	64	55
	Default	Cart PL PVDF Protein	Flask Normal	Fast Normal I
	1	None	Flask Prep Pump	Prep Pump
	2	None	Flask Blank	Fast Normal I
	3	Cart Begin	Flask Standard	Fast Normal I
GP PVDF Protein		48	64	55
	Default	Cart GP PVDF Protein	Flask Normal	Fast Normal I
	1	None	Flask Prep Pump	Prep Pump
	2	None	Flask Blank	Fast Normal I
	3	Cart Begin Gas-phase	Flask Standard	Fast Normal I
PL PVDF Peptide		48	64	55
	Default	Cart PL PVDF Peptide	Flask Normal	Fast Normal I
	1	None	Flask Prep Pump	Prep Pump
	2	None	Flask Blank	Fast Normal I
	3	Cart Begin	Flask Standard	Fast Normal I

Method Name		Cart. Temp °C	Flask Temp °C	Column Temp °C
GP PVDF Peptide		48	64	55
	Default	Cart GP PVDF Peptide	Flask Normal	Fast Normal I
	1	None	Flask Prep Pump	Prep Pump
	2	None	Flask Blank	Fast Normal I
	3	Cart Begin Gas-phase	Flask Standard	Fast Normal I
Run Gradient		35	64	55
	Default	None	Run Gradient	Fast Normal I
PTH-Standards		35	64	55
	Default	None	Flask Standard	Fast Normal I
Inject Optimize		35	64	55
	Default	None	Injector Optimize	Fast Normal I
Manual Injection		35	64	55
	Default	None	Manual Injection	Fast Normal I
Flask Optimize		45	64	55
	Default	Flask Optimize-Cart	Flask Optimize-Flsk	Fast Normal I

Gradient List

Gradient Tables

Fast Normal 1 Gradient

Gradient Name			Time (min)	%B	µL/min	Events
Fast Normal 1	**Max. Pressure:**	4000				
	Min. Pressure:	0	0	6	325	12
	Target Pressure:	1000	0.3	6	325	1
	Target Time:	1.0	0.4	16	325	1
	Data Collect Time:	20.0	18	45	325	1
			18.5	90	325	1
			19	90	325	1
			21.5	90	325	0

Prep Pump Gradient

Gradient Name			Time (min)	%B	µL/min	Events
Prep Pump	**Max. Pressure:**	4000				
	Min. Pressure:	0	0	50	325	0
	Target Pressure:	1500	20	50	325	0
	Target Time:	1.0				
	Data Collect Time:	10.0				

Procedure Listings

C

Overview

About This Appendix

All available procedure lists are in this appendix.

In This Chapter

This appendix contains the following topics:

Bottle Change Procedure List

Bottle Change for R1

Total run time: 1:35

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	261	Set for Bottle R1	0	N
3	303	Select Regulator	1	N
4	7	Vent R1	10	N
5	9	Backflush R1	20	N
6	260	Pause for Bottle Change	0	N
7	8	Flush R1	15	N
8	4	Del R1, Waste	10	N
9	135	Flush Cart Reagent Block	10	N
10	143	Wash Cart Reagent Block	10	N
11	135	Flush Cart Reagent Block	20	N
12	259	End	0	N

Bottle Change for R1-Leak

Total run time: 2:45

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	261	Set for Bottle R1	0	N
3	303	Select Regulator	1	N
4	304	Save Regulator Setpoint	0	N
5	7	Vent R1	10	N
6	9	Backflush R1	20	N
7	260	Pause for Bottle Change	0	N
8	305	Set Reg Setpoint (10th psi)	38	N
9	310	Set Tolerance (100th psi)	20	N
10	257	Wait	25	N
11	308	Close Pressure Valve	0	N
12	257	Wait	15	N
13	307	Compare Pressures (10th psi)	38	N
14	317	Save Regulator Pressure	0	N
15	310	Set Tolerance (100th psi)	5	N
16	257	Wait	30	N
17	318	Compare Saved Pressure	0	N
18	309	Restore Reg Setpoint	0	N
19	8	Flush R1	15	N
20	4	Del R1, Waste	10	N

Step	Function #	Function Name	Time (sec)	Global Time
21	135	Flush Cart Reagent Block	10	N
22	143	Wash Cart Reagent Block	10	N
23	135	Flush Cart Reagent Block	20	N
24	259	End	0	N

Bottle Change for R2 Total run time: 1:00

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	262	Set for Bottle R2	0	N
3	303	Select Regulator	2	N
4	17	Vent R2g	10	N
5	19	Backflush R2g	10	N
6	260	Pause for Bottle Change	0	N
7	18	Flush R2g	15	N
8	143	Wash Cart Reagent Block	5	N
9	135	Flush Cart Reagent Block	20	N
10	259	End	0	N

Bottle Change for R2-Leak

Total run time: 2:00

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	262	Set for Bottle R2	0	N
3	303	Select Regulator	2	N
4	304	Save Regulator Setpoint	0	N
5	17	Vent R2g	10	N
6	19	Backflush R2g	10	N
7	260	Pause for Bottle Change	0	N
8	305	Set Reg Setpoint (10th psi)	38	N
9	310	Set Tolerance (100th psi)	20	N
10	257	Wait	15	N
11	308	Close Pressure Valve	0	N
12	257	Wait	15	N
13	307	Compare Pressures (10th psi)	38	N
14	317	Save Regulator Pressure	0	N
15	310	Set Tolerance (100th psi)	5	N
16	257	Wait	30	N
17	318	Compare Saved Pressure	0	N
18	309	Restore Reg Setpoint	0	N
19	18	Flush R2g	15	N
20	143	Wash Cart Reagent Block	5	N
21	135	Flush Cart Reagent Block	20	N
22	259	End	0	N

Bottle Change for R3

Total run time: 3:35

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	263	Set for Bottle R3	0	N
3	303	Select Regulator	3	N
4	304	Save Regulator Setpoint	0	N
5	27	Vent R3	10	N
6	29	Backflush R3	60	N
7	39	Backflush R3g	20	N
8	260	Pause for Bottle Change	0	N
9	305	Set Reg Setpoint (10th psi)	15	N
10	28	Flush R3	15	N
11	24	Del R3, Waste	60	N
12	34	Del R3g, Waste	20	N

Step	Function #	Function Name	Time (sec)	Global Time
13	309	Restore Reg Setpoint	0	N
14	144	Wash Cart Solvent Block	10	N
15	136	Flush Cart Solvent Block	20	N
16	259	End	0	N

Bottle Change for R3-Leak

Total run time: 4:50

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	263	Set for Bottle R3	0	N
3	303	Select Regulator	3	N
4	304	Save Regulator Setpoint	0	N
5	27	Vent R3	10	N
6	29	Backflush R3	60	N
7	39	Backflush R3g	20	N
8	260	Pause for Bottle Change	0	N
9	305	Set Reg Setpoint (10th psi)	38	N
10	310	Set Tolerance (100th psi)	20	N
11	257	Wait	30	N
12	308	Close Pressure Valve	0	N
13	257	Wait	15	N
14	307	Compare Pressures (10th psi)	38	N
15	317	Save Regulator Pressure	0	N
16	310	Set Tolerance (100th psi)	5	N
17	257	Wait	30	N
18	318	Compare Saved Pressure	0	N
19	305	Set Reg Setpoint (10th psi)	15	N
20	28	Flush R3	15	N
21	24	Del R3, Waste	60	N
22	34	Del R3g, Waste	20	N
23	309	Restore Reg Setpoint	0	N
24	144	Wash Cart Solvent Block	10	N
25	136	Flush Cart Solvent Block	20	N
26	259	End	0	N

Bottle Change for R4 Total run time: 1:35

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	264	Set for Bottle R4	0	N
3	303	Select Regulator	6	N
4	154	Vent R4	10	N
5	156	Backflush R4	20	N
6	260	Pause for Bottle Change	0	N
7	155	Flush R4	15	N
8	157	Del R4, Waste	10	N
9	218	Flush Large Loop (Flask)	10	N
10	220	Wash Large Loop (Flask)	10	N
11	218	Flush Large Loop (Flask)	20	N
12	259	End	0	N

Bottle Change for R4-Leak Total run time 3:00

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	264	Set for Bottle R4	0	N
3	303	Select Regulator	6	N
4	304	Save Regulator Setpoint	0	N
5	154	Vent R4	10	N
6	156	Backflush R4	20	N
7	260	Pause for Bottle Change	0	N
8	305	Set Reg Setpoint (10th psi)	38	N
9	310	Set Tolerance (100th psi)	20	N
10	257	Wait	40	N
11	308	Close Pressure Valve	0	N
12	257	Wait	15	N
13	307	Compare Pressures (10th psi)	38	N
14	317	Save Regulator Pressure	0	N
15	310	Set Tolerance (100th psi)	5	N
16	257	Wait	30	N
17	318	Compare Saved Pressure	0	N
18	309	Restore Reg Setpoint	0	N
19	155	Flush R4	15	N
20	157	Del R4, Waste	10	N
21	218	Flush Large Loop (Flask)	10	N
22	220	Wash Large Loop (Flask)	10	N

Step	Function #	Function Name	Time (sec)	Global Time
23	218	Flush Large Loop (Flask)	20	N
24	259	End	0	N

Bottle Change for R5 Total run time: 1:25

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	265	Set for Bottle R5	0	N
3	303	Select Regulator	7	N
4	164	Vent R5	10	N
5	166	Backflush R5	15	N
6	260	Pause for Bottle Change	0	N
7	165	Flush R5	10	N
8	167	Del R5, Waste	10	N
9	218	Flush Large Loop (Flask)	10	N
10	220	Wash Large Loop (Flask)	10	N
11	218	Flush Large Loop (Flask)	20	N
12	259	End	0	N

Bottle Change for R5-Leak Total run time: 2:50

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	265	Set for Bottle R5	0	N
3	303	Select Regulator	7	N
4	304	Save Regulator Setpoint	0	N
5	164	Vent R5	10	N
6	166	Backflush R5	15	N
7	260	Pause for Bottle Change	0	N
8	305	Set Reg Setpoint (10th psi)	38	N
9	310	Set Tolerance (100th psi)	20	N
10	257	Wait	40	N
11	308	Close Pressure Valve	0	N
12	257	Wait	15	N
13	307	Compare Pressures (10th psi)	38	N
14	317	Save Regulator Pressure	0	N
15	310	Set Tolerance (100th psi)	5	N
16	257	Wait	30	N
17	318	Compare Saved Pressure	0	N

Step	Function #	Function Name	Time (sec)	Global Time
18	309	Restore Reg Setpoint	0	N
19	165	Flush R5	10	N
20	167	Del R5, Waste	10	N
21	218	Flush Large Loop (Flask)	10	N
22	220	Wash Large Loop (Flask)	10	N
23	218	Flush Large Loop (Flask)	20	N
24	259	End	0	N

Bottle Change for S1 Total run time: 1:30

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	266	Set for Bottle S1	0	N
3	303	Select Regulator	4	N
4	47	Vent S1	10	N
5	49	Backflush S1	20	N
6	260	Pause for Bottle Change	0	N
7	48	Flush S1	15	N
8	44	Del S1, Waste	10	N
9	136	Flush Cart Solvent Block	10	N
10	144	Wash Cart Solvent Block	5	N
11	136	Flush Cart Solvent Block	20	N
12	259	End	0	N

Bottle Change for S1-Leak Total run time: 2:55

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	266	Set for Bottle S1	0	N
3	303	Select Regulator	4	N
4	304	Save Regulator Setpoint	0	N
5	47	Vent S1	10	N
6	49	Backflush S1	20	N
7	260	Pause for Bottle Change	0	N
8	305	Set Reg Setpoint (10th psi)	38	N
9	310	Set Tolerance (100th psi)	20	N
10	257	Wait	40	N
11	308	Close Pressure Valve	0	N
12	257	Wait	15	N

Step	Function #	Function Name	Time (sec)	Global Time
13	307	Compare Pressures (10th psi)	38	N
14	317	Save Regulator Pressure	0	N
15	310	Set Tolerance (100th psi)	5	N
16	257	Wait	30	N
17	318	Compare Saved Pressure	0	N
18	309	Restore Reg Setpoint	0	N
19	48	Flush S1	15	N
20	44	Del S1, Waste	10	N
21	136	Flush Cart Solvent Block	10	N
22	144	Wash Cart Solvent Block	5	N
23	136	Flush Cart Solvent Block	20	N
24	259	End	0	N

Bottle Change for S2 Total run time: 1:15

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	267	Set for Bottle S2	0	N
3	303	Select Regulator	4	N
4	57	Vent S2	10	N
5	59	Backflush S2	20	N
6	260	Pause for Bottle Change	0	N
7	58	Flush S2	15	N
8	54	Del S2, Waste	10	N
9	136	Flush Cart Solvent Block	20	N
10	259	End	0	N

Bottle Change for S2-Leak Total run time: 2:40

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	267	Set for Bottle S2	0	N
3	303	Select Regulator	4	N
4	304	Save Regulator Setpoint	0	N
5	57	Vent S2	10	N
6	59	Backflush S2	20	N
7	260	Pause for Bottle Change	0	N
8	305	Set Reg Setpoint (10th psi)	38	N
9	310	Set Tolerance (100th psi)	20	N

Step	Function #	Function Name	Time (sec)	Global Time
10	257	Wait	40	N
11	308	Close Pressure Valve	0	N
12	257	Wait	15	N
13	307	Compare Pressures (10th psi)	38	N
14	317	Save Regulator Pressure	0	N
15	310	Set Tolerance (100th psi)	5	N
16	257	Wait	30	N
17	318	Compare Saved Pressure	0	N
18	309	Restore Reg Setpoint	0	N
19	58	Flush S2	15	N
20	54	Del S2, Waste	10	N
21	136	Flush Cart Solvent Block	20	N
22	259	End	0	N

Bottle Change for S3 Total run time: 1:15

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	268	Set for Bottle S3	0	N
3	303	Select Regulator	4	N
4	67	Vent S3	10	N
5	69	Backflush S3	20	N
6	260	Pause for Bottle Change	0	N
7	68	Flush S3	15	N
8	64	Del S3, Waste	10	N
9	136	Flush Cart Solvent Block	20	N
10	259	End	0	N

Bottle Change for S3-Leak Total run time: 2:40

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	268	Set for Bottle S3	0	N
3	303	Select Regulator	4	N
4	304	Save Regulator Setpoint	0	N
5	67	Vent S3	10	N
6	69	Backflush S3	20	N
7	260	Pause for Bottle Change	0	N
8	305	Set Reg Setpoint (10th psi)	38	N

Step	Function #	Function Name	Time (sec)	Global Time
9	310	Set Tolerance (100th psi)	20	N
10	257	Wait	40	N
11	308	Close Pressure Valve	0	N
12	257	Wait	15	N
13	307	Compare Pressures (10th psi)	38	N
14	317	Save Regulator Pressure	0	N
15	310	Set Tolerance (100th psi)	5	N
16	257	Wait	30	N
17	318	Compare Saved Pressure	0	N
18	309	Restore Reg Setpoint	0	N
19	68	Flush S3	15	N
20	64	Del S3, Waste	10	N
21	136	Flush Cart Solvent Block	20	N
22	259	End	0	N

Bottle Change for S4 Total run time: 1:15

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	269	Set for Bottle S4	0	N
3	303	Select Regulator	6	N
4	174	Vent S4	10	N
5	176	Backflush S4	20	N
6	260	Pause for Bottle Change	0	N
7	175	Flush S4	15	N
8	177	Del S4, Waste	10	N
9	218	Flush Large Loop (Flask)	20	N
10	259	End	0	N

Bottle Change for S4-Leak Total run time: 2:40

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	269	Set for Bottle S4	0	N
3	303	Select Regulator	6	N
4	304	Save Regulator Setpoint	0	N
5	174	Vent S4	10	N
6	176	Backflush S4	20	N
7	260	Pause for Bottle Change	0	N

Step	Function #	Function Name	Time (sec)	Global Time
8	305	Set Reg Setpoint (10th psi)	38	N
9	310	Set Tolerance (100th psi)	20	N
10	257	Wait	40	N
11	308	Close Pressure Valve	0	N
12	257	Wait	15	N
13	307	Compare Pressures (10th psi)	38	N
14	317	Save Regulator Pressure	0	N
15	310	Set Tolerance (100th psi)	5	N
16	257	Wait	30	N
17	318	Compare Saved Pressure	0	N
18	309	Restore Reg Setpoint	0	N
19	175	Flush S4	15	N
20	177	Del S4, Waste	10	N
21	218	Flush Large Loop (Flask)	20	N
22	259	End	0	N

Bottle Change for X1 Total run time: 3:25

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	270	Set for Bottle X1	0	N
3	303	Select Regulator	7	N
4	77	Vent X1	10	N
5	79	Backflush X1	60	N
6	89	Backflush X1g	20	N
7	260	Pause for Bottle Change	0	N
8	78	Flush X1	15	N
9	74	Del X1, Waste	60	N
10	84	Del X1g, Waste	10	N
11	143	Wash Cart Reagent Block	10	N
12	135	Flush Cart Reagent Block	20	N
13	259	End	0	N

Bottle Change for X1-Leak

Total run time: 4:50

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	270	Set for Bottle X1	0	N
3	303	Select Regulator	7	N
4	304	Save Regulator Setpoint	0	N
5	77	Vent X1	10	N
6	79	Backflush X1	60	N
7	89	Backflush X1g	20	N
8	260	Pause for Bottle Change	0	N
9	305	Set Reg Setpoint (10th psi)	38	N
10	310	Set Tolerance (100th psi)	20	N
11	257	Wait	40	N
12	308	Close Pressure Valve	0	N
13	257	Wait	15	N
14	307	Compare Pressures (10th psi)	38	N
15	317	Save Regulator Pressure	0	N
16	310	Set Tolerance (100th psi)	5	N
17	257	Wait	30	N
18	318	Compare Saved Pressure	0	N
19	309	Restore Reg Setpoint	0	N
20	78	Flush X1	15	N
21	74	Del X1, Waste	60	N
22	84	Del X1g, Waste	10	N
23	143	Wash Cart Reagent Block	10	N
24	135	Flush Cart Reagent Block	20	N
25	259	End	0	N

Bottle Change for X2

Total run time: 2:05

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	271	Set for Bottle X2	0	N
3	303	Select Regulator	7	N
4	184	Vent X2	10	N
5	186	Backflush X2	20	N
6	196	Backflush X2g	20	N
7	260	Pause for Bottle Change	0	N
8	185	Flush X2	15	N
9	187	Del X2, Waste	20	N

Step	Function #	Function Name	Time (sec)	Global Time
10	197	Del X2g, Waste	10	N
11	220	Wash Large Loop (Flask)	10	N
12	218	Flush Large Loop (Flask)	20	N
13	259	End	0	N

Bottle Change for X2-Leak

Total run time: 3:30

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	271	Set for Bottle X2	0	N
3	303	Select Regulator	7	N
4	304	Save Regulator Setpoint	0	N
5	184	Vent X2	10	N
6	186	Backflush X2	20	N
7	196	Backflush X2g	20	N
8	260	Pause for Bottle Change	0	N
9	305	Set Reg Setpoint (10th psi)	38	N
10	310	Set Tolerance (100th psi)	20	N
11	257	Wait	40	N
12	308	Close Pressure Valve	0	N
13	257	Wait	15	N
14	307	Compare Pressures (10th psi)	38	N
15	317	Save Regulator Pressure	0	N
16	310	Set Tolerance (100th psi)	5	N
17	257	Wait	30	N
18	318	Compare Saved Pressure	0	N
19	309	Restore Reg Setpoint	0	N
20	185	Flush X2	15	N
21	187	Del X2, Waste	20	N
22	197	Del X2g, Waste	10	N
23	220	Wash Large Loop (Flask)	10	N
24	218	Flush Large Loop (Flask)	20	N
25	259	End	0	N

Bottle Change for X3 Both

Total run time: 5:45

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	272	Set for Bottle X3	0	N

Step	Function #	Function Name	Time (sec)	Global Time
3	303	Select Regulator	8	N
4	97	Vent X3, Cart	10	N
5	99	Backflush X3, Cart	60	N
6	206	Backflush X3, Flask	60	N
7	260	Pause for Bottle Change	0	N
8	98	Flush X3, Cart	15	N
9	94	Del X3, Waste	60	N
10	135	Flush Cart Reagent Block	10	N
11	143	Wash Cart Reagent Block	10	N
12	135	Flush Cart Reagent Block	20	N
13	207	Del X3, Waste, Flask	60	N
14	218	Flush Large Loop (Flask)	10	N
15	220	Wash Large Loop (Flask)	10	N
16	218	Flush Large Loop (Flask)	20	N
17	259	End	0	N

Bottle Change for X3 Both-Leak

Total run time: 7:10

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	272	Set for Bottle X3	0	N
3	303	Select Regulator	8	N
4	304	Save Regulator Setpoint	0	N
5	97	Vent X3, Cart	10	N
6	99	Backflush X3, Cart	60	N
7	206	Backflush X3, Flask	60	N
8	260	Pause for Bottle Change	0	N
9	305	Set Reg Setpoint (10th psi)	38	N
10	310	Set Tolerance (100th psi)	20	N
11	257	Wait	40	N
12	308	Close Pressure Valve	0	N
13	257	Wait	15	N
14	307	Compare Pressures (10th psi)	38	N
15	317	Save Regulator Pressure	0	N
16	310	Set Tolerance (100th psi)	5	N
17	257	Wait	30	N
18	318	Compare Saved Pressure	0	N
19	309	Restore Reg Setpoint	0	N
20	98	Flush X3, Cart	15	N
21	94	Del X3, Waste	60	N

Step	Function #	Function Name	Time (sec)	Global Time
22	135	Flush Cart Reagent Block	10	N
23	143	Wash Cart Reagent Block	10	N
24	135	Flush Cart Reagent Block	20	N
25	207	Del X3, Waste, Flask	60	N
26	218	Flush Large Loop (Flask)	10	N
27	220	Wash Large Loop (Flask)	10	N
28	218	Flush Large Loop (Flask)	20	N
29	259	End	0	N

Cleanup Procedure List

Delivery Line Backflush

Total run time: 16:25

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	7	Vent R1	5	N
3	9	Backflush R1	60	N
4	17	Vent R2g	10	N
5	19	Backflush R2g	60	N
6	27	Vent R3	5	N
7	29	Backflush R3	60	N
8	39	Backflush R3g	60	N
9	47	Vent S1	5	N
10	49	Backflush S1	60	N
11	59	Backflush S2	60	N
12	69	Backflush S3	60	N
13	77	Vent X1	5	N
14	79	Backflush X1	60	N
15	89	Backflush X1g	60	N
16	97	Vent X3, Cart	5	N
17	99	Backflush X3, Cart	60	N
18	206	Backflush X3, Flask	60	N
19	154	Vent R4	5	N
20	156	Backflush R4	60	N
21	164	Vent R5	5	N
22	166	Backflush R5	30	N
23	174	Vent S4	5	N
24	176	Backflush S4	60	N
25	184	Vent X2	5	N
26	186	Backflush X2	60	N
27	196	Backflush X2g	60	N
28	259	End	0	N

Cartridge Valve Block Wash - S1

Total run time: 12:56

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	103	Wash Reagent Block (S1)	180	N
3	104	Wash Solvent Block (S1)	180	N
4	101	Wash Input Block (S1)	180	N

Step	Function #	Function Name	Time (sec)	Global Time
5	123	Select Cartridge A	0	N
6	102	Wash Output Block (S1)	180	N
7	41	Del S1, Cart (top)	120	N
8	42	Del S1, Cart (bottom)	10	N
9	41	Del S1, Cart (top)	10	N
10	42	Del S1, Cart (bottom)	10	N
11	41	Del S1, Cart (top)	10	N
12	42	Del S1, Cart (bottom)	10	N
13	41	Del S1, Cart (top)	10	N
14	42	Del S1, Cart (bottom)	10	N
15	41	Del S1, Cart (top)	60	N
16	148	Cartridge Wait	30	N
17	41	Del S1, Cart (top)	60	N
18	148	Cartridge Wait	30	N
19	42	Del S1, Cart (bottom)	60	N
20	147	End Cartridge Select	0	N
21	124	Select Cartridge B	0	N
22	257	Wait	120	N
23	41	Del S1, Cart (top)	120	N
24	42	Del S1, Cart (bottom)	10	N
25	41	Del S1, Cart (top)	10	N
26	42	Del S1, Cart (bottom)	10	N
27	41	Del S1, Cart (top)	10	N
28	42	Del S1, Cart (bottom)	10	N
29	41	Del S1, Cart (top)	10	N
30	42	Del S1, Cart (bottom)	10	N
31	41	Del S1, Cart (top)	60	N
32	148	Cartridge Wait	30	N
33	41	Del S1, Cart (top)	60	N
34	148	Cartridge Wait	30	N
35	42	Del S1, Cart (bottom)	60	N
36	147	End Cartridge Select	0	N
37	125	Select Cartridge C	0	N
38	257	Wait	120	N
39	41	Del S1, Cart (top)	120	N
40	42	Del S1, Cart (bottom)	10	N
41	41	Del S1, Cart (top)	10	N
42	42	Del S1, Cart (bottom)	10	N
43	41	Del S1, Cart (top)	10	N
44	42	Del S1, Cart (bottom)	10	N
45	41	Del S1, Cart (top)	10	N

Step	Function #	Function Name	Time (sec)	Global Time
46	42	Del S1, Cart (bottom)	10	N
47	41	Del S1, Cart (top)	60	N
48	148	Cartridge Wait	30	N
49	41	Del S1, Cart (top)	60	N
50	148	Cartridge Wait	30	N
51	42	Del S1, Cart (bottom)	60	N
52	147	End Cartridge Select	0	N
53	126	Select Cartridge D	0	N
54	257	Wait	120	N
55	41	Del S1, Cart (top)	120	N
56	42	Del S1, Cart (bottom)	10	N
57	41	Del S1, Cart (top)	10	N
58	42	Del S1, Cart (bottom)	10	N
59	41	Del S1, Cart (top)	10	N
60	42	Del S1, Cart (bottom)	10	N
61	41	Del S1, Cart (top)	10	N
62	42	Del S1, Cart (bottom)	10	N
63	41	Del S1, Cart (top)	60	N
64	148	Cartridge Wait	30	N
65	41	Del S1, Cart (top)	60	N
66	148	Cartridge Wait	30	N
67	42	Del S1, Cart (bottom)	60	N
68	147	End Cartridge Select	0	N
69	135	Flush Cart Reagent Block	90	N
70	136	Flush Cart Solvent Block	90	N
71	137	Flush Input Block	90	N
72	138	Flush Output Block	90	N
73	139	Flush Small Loop (Cart)	90	N
74	140	Flush Large Loop (Cart)	90	N
75	123	Select Cartridge A	0	N
76	131	Dry Cart (top)	90	N
77	147	End Cartridge Select	0	N
78	124	Select Cartridge B	0	N
79	131	Dry Cart (top)	90	N
80	147	End Cartridge Select	0	N
81	125	Select Cartridge C	0	N
82	131	Dry Cart (top)	90	N
83	147	End Cartridge Select	0	N
84	126	Select Cartridge D	0	N
85	131	Dry Cart (top)	90	N
86	147	End Cartridge Select	0	N

Step	Function #	Function Name	Time (sec)	Global Time
87	259	End	0	N

System Clean-Out - X3

Total run time: 26:44

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	223	Inject Position	0	N
3	303	Select Regulator	1	N
4	305	Set Reg Setpoint (10th psi)	0	N
5	303	Select Regulator	2	N
6	305	Set Reg Setpoint (10th psi)	0	N
7	303	Select Regulator	3	N
8	305	Set Reg Setpoint (10th psi)	0	N
9	303	Select Regulator	4	N
10	305	Set Reg Setpoint (10th psi)	0	N
11	303	Select Regulator	5	N
12	305	Set Reg Setpoint (10th psi)	45	N
13	303	Select Regulator	6	N
14	305	Set Reg Setpoint (10th psi)	0	N
15	303	Select Regulator	7	N
16	305	Set Reg Setpoint (10th psi)	0	N
17	303	Select Regulator	8	N
18	305	Set Reg Setpoint (10th psi)	45	N
19	94	Del X3, Waste	30	N
20	135	Flush Cart Reagent Block	5	N
21	341	X3 to X1	60	N
22	135	Flush Cart Reagent Block	5	N
23	333	X3 to R1	60	N
24	135	Flush Cart Reagent Block	5	N
25	286	X3 to R2	60	N
26	19	Backflush R2g	10	N
27	287	X3 to R3g	120	N
28	39	Backflush R3g	10	N
29	342	X3 to X2	30	N
30	288	X3 to X1g	30	N
31	89	Backflush X1g	10	N
32	334	X3 to R3	60	N
33	337	X3 to S1	60	N
34	338	X3 to S2	60	N
35	339	X3 to S3	60	N

Step	Function #	Function Name	Time (sec)	Global Time
36	96	Load X3, Cart (lg loop)	60	N
37	140	Flush Large Loop (Cart)	5	N
38	95	Load X3, Cart (sm loop)	60	N
39	139	Flush Small Loop (Cart)	5	N
40	140	Flush Large Loop (Cart)	5	N
41	123	Select Cartridge A	0	N
42	257	Wait	1	N
43	343	X3 to Cart A (bottom)	60	N
44	132	Dry Cart (bottom)	10	N
45	124	Select Cartridge B	0	N
46	257	Wait	1	N
47	118	Transfer to Flask (X3)	30	N
48	121	Transfer to Flask (gas)	10	N
49	215	Empty Flask	10	N
50	125	Select Cartridge C	0	N
51	257	Wait	1	N
52	118	Transfer to Flask (X3)	30	N
53	131	Dry Cart (top)	10	N
54	126	Select Cartridge D	0	N
55	257	Wait	1	N
56	118	Transfer to Flask (X3)	30	N
57	121	Transfer to Flask (gas)	10	N
58	147	End Cartridge Select	0	N
59	136	Flush Cart Solvent Block	5	N
60	138	Flush Output Block	5	N
61	336	X3 to R5	30	N
62	218	Flush Large Loop (Flask)	0	N
63	335	X3 to R4	60	N
64	218	Flush Large Loop (Flask)	0	N
65	202	Load X3, Flask (sm loop)	60	N
66	217	Flush Small Loop (Flask)	5	N
67	289	X3 to X2g	60	N
68	196	Backflush X2g	10	N
69	203	Load X3, Flask (lg loop)	60	N
70	218	Flush Large Loop (Flask)	5	N
71	340	X3 to S4	30	N
72	213	Dry Flask	5	N
73	215	Empty Flask	10	N
74	118	Transfer to Flask (X3)	30	N
75	121	Transfer to Flask (gas)	10	N
76	222	Flush Flask/Injector	10	N

Step	Function #	Function Name	Time (sec)	Global Time
77	226	Load Position	0	N
78	201	Del X3, Flask	30	N
79	213	Dry Flask	5	N
80	222	Flush Flask/Injector	10	N
81	9	Backflush R1	10	N
82	19	Backflush R2g	10	N
83	29	Backflush R3	10	N
84	49	Backflush S1	10	N
85	59	Backflush S2	10	N
86	69	Backflush S3	10	N
87	79	Backflush X1	10	N
88	97	Vent X3, Cart	5	N
89	99	Backflush X3, Cart	10	N
90	206	Backflush X3, Flask	10	N
91	156	Backflush R4	15	N
92	166	Backflush R5	10	N
93	176	Backflush S4	10	N
94	186	Backflush X2	10	N
95	196	Backflush X2g	10	N
96	259	End	0	N

System Flush - Argon

Total run time: 75:00

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	303	Select Regulator	1	N
3	305	Set Reg Setpoint (10th psi)	20	N
4	303	Select Regulator	2	N
5	305	Set Reg Setpoint (10th psi)	10	N
6	303	Select Regulator	3	N
7	305	Set Reg Setpoint (10th psi)	30	N
8	303	Select Regulator	4	N
9	305	Set Reg Setpoint (10th psi)	17	N
10	303	Select Regulator	5	N
11	305	Set Reg Setpoint (10th psi)	50	N
12	303	Select Regulator	6	N
13	305	Set Reg Setpoint (10th psi)	35	N
14	303	Select Regulator	7	N
15	305	Set Reg Setpoint (10th psi)	20	N
16	303	Select Regulator	8	N

Step	Function #	Function Name	Time (sec)	Global Time
17	305	Set Reg Setpoint (10th psi)	50	N
18	226	Load Position	0	N
19	9	Backflush R1	180	N
20	19	Backflush R2g	180	N
21	29	Backflush R3	180	N
22	39	Backflush R3g	180	N
23	49	Backflush S1	180	N
24	59	Backflush S2	180	N
25	69	Backflush S3	180	N
26	79	Backflush X1	180	N
27	89	Backflush X1g	180	N
28	99	Backflush X3, Cart	180	N
29	206	Backflush X3, Flask	180	N
30	156	Backflush R4	180	N
31	166	Backflush R5	180	N
32	176	Backflush S4	180	N
33	186	Backflush X2	180	N
34	196	Backflush X2g	180	N
35	135	Flush Cart Reagent Block	180	N
36	136	Flush Cart Solvent Block	180	N
37	123	Select Cartridge A	0	N
38	121	Transfer to Flask (gas)	120	N
39	132	Dry Cart (bottom)	120	N
40	124	Select Cartridge B	0	N
41	121	Transfer to Flask (gas)	120	N
42	125	Select Cartridge C	0	N
43	131	Dry Cart (top)	120	N
44	126	Select Cartridge D	0	N
45	131	Dry Cart (top)	120	N
46	147	End Cartridge Select	0	N
47	137	Flush Input Block	60	N
48	138	Flush Output Block	60	N
49	139	Flush Small Loop (Cart)	60	N
50	140	Flush Large Loop (Cart)	60	N
51	214	Dry Flask (h press)	120	N
52	217	Flush Small Loop (Flask)	60	N
53	218	Flush Large Loop (Flask)	60	N
54	222	Flush Flask/Injector	60	N
55	223	Inject Position	0	N
56	222	Flush Flask/Injector	60	N
57	215	Empty Flask	60	N

Step	Function #	Function Name	Time (sec)	Global Time
58	303	Select Regulator	5	N
59	305	Set Reg Setpoint (10th psi)	35	N
60	303	Select Regulator	8	N
61	305	Set Reg Setpoint (10th psi)	30	N
62	259	End	0	N

Electrical Test Procedure

Electrical Test

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	311	Test Valves	2	N
3	312	Test Heaters	2	N
4	313	Test Pressure Board	2	N
5	314	Test 12-Bit A/D	2	N
6	315	Test 24-Bit A/D	2	N
7	316	Test Rheodyne	2	N
8	259	End	0	N

Flow Procedure List

Flask Optimization Total run time: 17:25

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	123	Select Cartridge A	0	N
3	121	Transfer to Flask (gas)	30	N
4	215	Empty Flask	15	N
5	173	Load S4, Flask (lg loop)	15	N
6	213	Dry Flask	10	N
7	218	Flush Large Loop (Flask)	10	N
8	63	Del S3, Cart (sensor)	15	N
9	257	Wait	0	N
10	121	Transfer to Flask (gas)	45	N
11	212	Bubble Flask	40	N
12	53	Del S2, Cart (sensor)	15	N
13	121	Transfer to Flask (gas)	45	N
14	212	Bubble Flask	40	N
15	213	Dry Flask	120	N
16	301	Pause	0	N
17	236	Pre-Conversion Dry	100	Y
18	301	Pause	0	N
19	218	Flush Large Loop (Flask)	10	N
20	153	Load R4, Flask (lg loop)	15	N
21	213	Dry Flask	10	N
22	218	Flush Large Loop (Flask)	10	N
23	173	Load S4, Flask (lg loop)	15	N
24	218	Flush Large Loop (Flask)	10	N
25	257	Wait	60	N
26	213	Dry Flask	300	N
27	301	Pause	0	N
28	237	Post-Conversion Dry	100	Y
29	215	Empty Flask	15	N
30	147	End Cartridge Select	0	N
30	259	End	0	N

Sensor & Delivery Test

Total run time: 25:01

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	226	Load Position	1	N
3	139	Flush Small Loop (Cart)	10	N
4	5	Load R1, Cart (sm loop)	18	N
5	139	Flush Small Loop (Cart)	10	N
6	143	Wash Cart Reagent Block	10	N
7	145	Wash Small Loop (Cart)	10	N
8	135	Flush Cart Reagent Block	20	N
9	139	Flush Small Loop (Cart)	20	N
10	140	Flush Large Loop (Cart)	10	N
11	76	Load X1, Cart (lg loop)	65	N
12	140	Flush Large Loop (Cart)	20	N
13	135	Flush Cart Reagent Block	20	N
14	303	Select Regulator	3	N
15	304	Save Regulator Setpoint	0	N
16	305	Set Reg Setpoint (10th psi)	15	N
17	26	Load R3, Cart (lg loop)	45	N
18	309	Restore Reg Setpoint	0	N
19	34	Del R3g, Waste	15	N
20	136	Flush Cart Solvent Block	10	N
21	140	Flush Large Loop (Cart)	10	N
22	144	Wash Cart Solvent Block	10	N
23	143	Wash Cart Reagent Block	15	N
24	111	Wash Input Block (S3)	10	N
25	107	Wash Output Block (S2)	10	N
26	146	Wash Large Loop (Cart)	10	N
27	135	Flush Cart Reagent Block	60	N
28	137	Flush Input Block	60	N
29	138	Flush Output Block	60	N
30	140	Flush Large Loop (Cart)	30	N
31	136	Flush Cart Solvent Block	60	N
32	123	Select Cartridge A	1	N
33	43	Del S1, Cart (sensor)	13	N
34	131	Dry Cart (top)	60	N
35	53	Del S2, Cart (sensor)	13	N
36	131	Dry Cart (top)	60	N
37	63	Del S3, Cart (sensor)	13	N
38	131	Dry Cart (top)	60	N
39	118	Transfer to Flask (X3)	50	N

Step	Function #	Function Name	Time (sec)	Global Time
40	121	Transfer to Flask (gas)	45	N
41	124	Select Cartridge B	1	N
42	53	Del S2, Cart (sensor)	13	N
43	131	Dry Cart (top)	60	N
44	125	Select Cartridge C	1	N
45	63	Del S3, Cart (sensor)	13	N
46	131	Dry Cart (top)	60	N
47	126	Select Cartridge D	1	N
48	63	Del S3, Cart (sensor)	13	N
49	131	Dry Cart (top)	60	N
50	147	End Cartridge Select	0	N
51	212	Bubble Flask	5	N
52	215	Empty Flask	20	N
53	221	Flush Injector	20	N
54	152	Load R4, Flask (sm loop)	18	N
55	213	Dry Flask	5	N
56	217	Flush Small Loop (Flask)	10	N
57	162	Load R5, Flask (sm loop)	18	N
58	213	Dry Flask	5	N
59	217	Flush Small Loop (Flask)	10	N
60	202	Load X3, Flask (sm loop)	35	N
61	213	Dry Flask	5	N
62	217	Flush Small Loop (Flask)	10	N
63	182	Load X2, Flask (sm loop)	35	N
64	213	Dry Flask	5	N
65	217	Flush Small Loop (Flask)	10	N
66	215	Empty Flask	10	N
67	218	Flush Large Loop (Flask)	10	N
68	173	Load S4, Flask (lg loop)	18	N
69	213	Dry Flask	5	N
70	218	Flush Large Loop (Flask)	10	N
71	173	Load S4, Flask (lg loop)	18	N
72	213	Dry Flask	5	N
73	218	Flush Large Loop (Flask)	10	N
74	221	Flush Injector	20	N
75	257	Wait	2	N
76	225	Load Injector	10	N
77	222	Flush Flask/Injector	20	N
78	259	End	0	N

Idle Procedure

Idle Total run time: 1:33

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	8	Flush R1	5	N
3	18	Flush R2g	10	N
4	28	Flush R3	5	N
5	48	Flush S1	10	N
6	58	Flush S2	15	N
7	68	Flush S3	10	N
8	78	Flush X1	5	N
9	98	Flush X3, Cart	10	N
10	155	Flush R4	5	N
11	165	Flush R5	3	N
12	175	Flush S4	10	N
13	185	Flush X2	5	N
14	259	End	0	N

Init Sensor Procedure

Init Sensor Procedure

Total run time: 9:16

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	145	Wash Small Loop (Cart)	10	N
3	139	Flush Small Loop (Cart)	30	N
4	273	Init Sm Loop Snsr, Cart	0	N
5	146	Wash Large Loop (Cart)	10	N
6	140	Flush Large Loop (Cart)	30	N
7	274	Init Lg Loop Snsr, Cart	0	N
8	107	Wash Output Block (S2)	15	N
9	112	Wash Output Block (S3)	15	N
10	138	Flush Output Block	40	N
11	123	Select Cartridge A	0	N
12	132	Dry Cart (bottom)	30	N
13	275	Init Cart A Snsr	0	N
14	147	End Cartridge Select	0	N
15	124	Select Cartridge B	0	N
16	132	Dry Cart (bottom)	30	N
17	276	Init Cart B Snsr	0	N
18	147	End Cartridge Select	0	N
19	125	Select Cartridge C	0	N
20	132	Dry Cart (bottom)	30	N
21	277	Init Cart C Snsr	0	N
22	147	End Cartridge Select	0	N
23	126	Select Cartridge D	0	N
24	132	Dry Cart (bottom)	30	N
25	278	Init Cart D Snsr	0	N
26	147	End Cartridge Select	0	N
27	149	Wash Transfer Line (S2)	40	N
28	141	Flush Transfer Line	40	N
29	279	Init Transfer Snsr	0	N
30	215	Empty Flask	60	N
32	219	Wash Small Loop (Flask)	10	N
33	217	Flush Small Loop (Flask)	30	N
34	280	Init Sm Loop Snsr, Flask	0	N
35	220	Wash Large Loop (Flask)	10	N
36	218	Flush Large Loop (Flask)	30	N
37	281	Init Lg Loop Snsr, Flask	0	N
38	226	Load Position	1	N

Step	Function #	Function Name	Time (sec)	Global Time
39	221	Flush Injector	30	N
40	257	Wait	5	N
41	221	Flush Injector	30	N
42	282	Init Injector Load Snsr	0	N
43	283	Init Injector Full Snsr	0	N
44	259	End	0	N

Leak Procedure List

Cartridge A Leak Test

Total run time: 1:36

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	303	Select Regulator	5	N
3	304	Save Regulator Setpoint	0	N
4	123	Select Cartridge A	0	N
5	131	Dry Cart (top)	10	N
6	257	Wait	1	N
7	305	Set Reg Setpoint (10th psi)	35	N
8	310	Set Tolerance (100th psi)	20	N
9	129	Pressurize Cart, top	30	N
10	308	Close Pressure Valve	1	N
11	129	Pressurize Cart, top	15	N
12	307	Compare Pressures (10th psi)	35	N
13	317	Save Regulator Pressure	0	N
14	310	Set Tolerance (100th psi)	10	N
15	129	Pressurize Cart, top	30	N
16	318	Compare Saved Pressure	0	N
17	309	Restore Reg Setpoint	0	N
18	131	Dry Cart (top)	10	N
19	147	End Cartridge Select	0	N
20	259	End	0	N

Cartridge B Leak Test

Total run time: 1:36

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	303	Select Regulator	5	N
3	304	Save Regulator Setpoint	0	N
4	124	Select Cartridge B	0	N
5	131	Dry Cart (top)	10	N
6	257	Wait	1	N
7	305	Set Reg Setpoint (10th psi)	35	N
8	310	Set Tolerance (100th psi)	20	N
9	129	Pressurize Cart, top	30	N
10	308	Close Pressure Valve	1	N
11	129	Pressurize Cart, top	15	N
12	307	Compare Pressures (10th psi)	35	N

Step	Function #	Function Name	Time (sec)	Global Time
13	317	Save Regulator Pressure	0	N
14	310	Set Tolerance (100th psi)	10	N
15	129	Pressurize Cart, top	30	N
16	318	Compare Saved Pressure	0	N
17	309	Restore Reg Setpoint	0	N
18	131	Dry Cart (top)	10	N
19	147	End Cartridge Select	0	N
20	259	End	0	N

Cartridge C Leak Test

Total run time: 1:36

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	303	Select Regulator	5	N
3	304	Save Regulator Setpoint	0	N
4	125	Select Cartridge C	0	N
5	131	Dry Cart (top)	10	N
6	257	Wait	1	N
7	305	Set Reg Setpoint (10th psi)	35	N
8	310	Set Tolerance (100th psi)	20	N
9	129	Pressurize Cart, top	30	N
10	308	Close Pressure Valve	1	N
11	129	Pressurize Cart, top	15	N
12	307	Compare Pressures (10th psi)	35	N
13	317	Save Regulator Pressure	0	N
14	310	Set Tolerance (100th psi)	10	N
15	129	Pressurize Cart, top	30	N
16	318	Compare Saved Pressure	0	N
17	309	Restore Reg Setpoint	0	N
18	131	Dry Cart (top)	10	N
19	147	End Cartridge Select	0	N
20	259	End	0	N

Cartridge D Leak Test

Total run time: 1:36

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	303	Select Regulator	5	N
3	304	Save Regulator Setpoint	0	N

Step	Function #	Function Name	Time (sec)	Global Time
4	126	Select Cartridge D	0	N
5	131	Dry Cart (top)	10	N
6	257	Wait	1	N
7	305	Set Reg Setpoint (10th psi)	35	N
8	310	Set Tolerance (100th psi)	20	N
9	129	Pressurize Cart, top	30	N
10	308	Close Pressure Valve	1	N
11	129	Pressurize Cart, top	15	N
12	307	Compare Pressures (10th psi)	35	N
13	317	Save Regulator Pressure	0	N
14	310	Set Tolerance (100th psi)	10	N
15	129	Pressurize Cart, top	30	N
16	318	Compare Saved Pressure	0	N
17	309	Restore Reg Setpoint	0	N
18	131	Dry Cart (top)	10	N
19	147	End Cartridge Select	0	N
20	259	End	0	N

R1 Leak Test Total run time: 2:55

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	303	Select Regulator	1	N
3	304	Save Regulator Setpoint	0	N
4	7	Vent R1	10	N
5	9	Backflush R1	20	N
6	305	Set Reg Setpoint (10th psi)	38	N
7	310	Set Tolerance (100th psi)	20	N
8	257	Wait	25	N
9	308	Close Pressure Valve	1	N
10	257	Wait	15	N
11	307	Compare Pressures (10th psi)	38	N
12	317	Save Regulator Pressure	0	N
13	310	Set Tolerance (100th psi)	5	N
14	257	Wait	30	N
15	318	Compare Saved Pressure	0	N
16	7	Vent R1	10	N
17	310	Set Tolerance (100th psi)	10	N
18	307	Compare Pressures (10th psi)	0	N
19	309	Restore Reg Setpoint	0	N

Step	Function #	Function Name	Time (sec)	Global Time
20	8	Flush R1	5	N
21	4	Del R1, Waste	10	N
22	135	Flush Cart Reagent Block	10	N
23	143	Wash Cart Reagent Block	10	N
24	135	Flush Cart Reagent Block	30	N
25	259	End	0	N

R2 Leak Test Total run time: 1:40

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	303	Select Regulator	2	N
3	304	Save Regulator Setpoint	0	N
4	305	Set Reg Setpoint (10th psi)	38	N
5	310	Set Tolerance (100th psi)	20	N
6	257	Wait	15	N
7	308	Close Pressure Valve	1	N
8	257	Wait	15	N
9	307	Compare Pressures (10th psi)	38	N
10	317	Save Regulator Pressure	0	N
11	310	Set Tolerance (100th psi)	5	N
12	257	Wait	30	N
13	318	Compare Saved Pressure	0	N
14	17	Vent R2g	35	N
15	310	Set Tolerance (100th psi)	10	N
16	307	Compare Pressures (10th psi)	0	N
17	309	Restore Reg Setpoint	0	N
18	18	Flush R2g	5	N
19	259	End	0	N

R3 Leak Test Total run time: 3:55

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	303	Select Regulator	3	N
3	304	Save Regulator Setpoint	0	N
4	27	Vent R3	10	N
5	29	Backflush R3	20	N
6	305	Set Reg Setpoint (10th psi)	38	N

Step	Function #	Function Name	Time (sec)	Global Time
7	310	Set Tolerance (100th psi)	20	N
8	257	Wait	30	N
9	308	Close Pressure Valve	1	N
10	257	Wait	15	N
11	307	Compare Pressures (10th psi)	38	N
12	317	Save Regulator Pressure	0	N
13	310	Set Tolerance (100th psi)	5	N
14	257	Wait	30	N
15	318	Compare Saved Pressure	0	N
16	27	Vent R3	15	N
17	310	Set Tolerance (100th psi)	10	N
18	307	Compare Pressures (10th psi)	0	N
19	305	Set Reg Setpoint (10th psi)	15	N
20	28	Flush R3	5	N
21	24	Del R3, Waste	60	N
22	136	Flush Cart Solvent Block	10	N
23	144	Wash Cart Solvent Block	10	N
24	136	Flush Cart Solvent Block	30	N
25	309	Restore Reg Setpoint	0	N
26	259	End	0	N

S1, S2, S3 Leak Test Total run time: 7:45

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	303	Select Regulator	4	N
3	304	Save Regulator Setpoint	0	N
4	47	Vent S1	5	N
5	49	Backflush S1	10	N
6	59	Backflush S2	10	N
7	69	Backflush S3	10	N
8	305	Set Reg Setpoint (10th psi)	38	N
9	310	Set Tolerance (100th psi)	20	N
10	257	Wait	40	N
11	308	Close Pressure Valve	1	N
12	257	Wait	15	N
13	307	Compare Pressures (10th psi)	38	N
14	317	Save Regulator Pressure	0	N
15	310	Set Tolerance (100th psi)	5	N
16	257	Wait	30	N

Step	Function #	Function Name	Time (sec)	Global Time
17	318	Compare Saved Pressure	0	N
18	47	Vent S1	60	N
19	310	Set Tolerance (100th psi)	10	N
20	307	Compare Pressures (10th psi)	0	N
21	305	Set Reg Setpoint (10th psi)	38	N
22	257	Wait	30	N
23	308	Close Pressure Valve	1	N
24	57	Vent S2	75	N
25	307	Compare Pressures (10th psi)	0	N
26	305	Set Reg Setpoint (10th psi)	38	N
27	257	Wait	30	N
28	308	Close Pressure Valve	1	N
29	67	Vent S3	60	N
30	307	Compare Pressures (10th psi)	0	N
31	309	Restore Reg Setpoint	0	N
32	48	Flush S1	10	N
33	44	Del S1, Waste	10	N
34	136	Flush Cart Solvent Block	10	N
35	64	Del S3, Waste	10	N
36	136	Flush Cart Solvent Block	10	N
37	54	Del S2, Waste	10	N
38	136	Flush Cart Solvent Block	30	N
39	259	End	0	N

R4, S4 Leak Test Total run time: 4:35

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	303	Select Regulator	6	N
3	304	Save Regulator Setpoint	0	N
4	174	Vent S4	10	N
5	156	Backflush R4	20	N
6	176	Backflush S4	20	N
7	305	Set Reg Setpoint (10th psi)	38	N
8	310	Set Tolerance (100th psi)	20	N
9	257	Wait	40	N
10	308	Close Pressure Valve	1	N
11	257	Wait	15	N
12	307	Compare Pressures (10th psi)	38	N
13	317	Save Regulator Pressure	0	N

Step	Function #	Function Name	Time (sec)	Global Time
14	310	Set Tolerance (100th psi)	5	N
15	257	Wait	30	N
16	318	Compare Saved Pressure	0	N
17	154	Vent R4	15	N
18	310	Set Tolerance (100th psi)	10	N
19	307	Compare Pressures (10th psi)	0	N
20	305	Set Reg Setpoint (10th psi)	38	N
21	257	Wait	30	N
22	308	Close Pressure Valve	1	N
23	174	Vent S4	25	N
24	307	Compare Pressures (10th psi)	0	N
25	309	Restore Reg Setpoint	0	N
26	157	Del R4, Waste	10	N
27	218	Flush Large Loop (Flask)	10	N
28	220	Wash Large Loop (Flask)	20	N
29	218	Flush Large Loop (Flask)	30	N
30	259	End	0	N

R5, X1, X2 Leak Test Total run time: 7:15

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	303	Select Regulator	7	N
3	304	Save Regulator Setpoint	0	N
4	77	Vent X1	5	N
5	79	Backflush X1	10	N
6	186	Backflush X2	10	N
7	166	Backflush R5	5	N
8	305	Set Reg Setpoint (10th psi)	38	N
9	310	Set Tolerance (100th psi)	20	N
10	257	Wait	40	N
11	308	Close Pressure Valve	1	N
12	257	Wait	15	N
13	307	Compare Pressures (10th psi)	38	N
14	317	Save Regulator Pressure	0	N
15	310	Set Tolerance (100th psi)	5	N
16	257	Wait	30	N
17	318	Compare Saved Pressure	0	N
18	164	Vent R5	15	N
19	310	Set Tolerance (100th psi)	10	N

Step	Function #	Function Name	Time (sec)	Global Time
20	307	Compare Pressures (10th psi)	0	N
21	305	Set Reg Setpoint (10th psi)	38	N
22	257	Wait	30	N
23	308	Close Pressure Valve	1	N
24	77	Vent X1	15	N
25	307	Compare Pressures (10th psi)	0	N
26	305	Set Reg Setpoint (10th psi)	38	N
27	257	Wait	30	N
28	308	Close Pressure Valve	1	N
29	184	Vent X2	25	N
30	307	Compare Pressures (10th psi)	0	N
31	309	Restore Reg Setpoint	0	N
32	185	Flush X2	5	N
33	74	Del X1, Waste	70	N
34	135	Flush Cart Reagent Block	10	N
35	143	Wash Cart Reagent Block	10	N
36	135	Flush Cart Reagent Block	30	N
37	187	Del X2, Waste	15	N
38	218	Flush Large Loop (Flask)	10	N
39	167	Del R5, Waste	5	N
40	218	Flush Large Loop (Flask)	10	N
41	220	Wash Large Loop (Flask)	10	N
42	218	Flush Large Loop (Flask)	30	N
43	259	End	0	N

X3 Leak Test Total run time: 5:15

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	303	Select Regulator	8	N
3	304	Save Regulator Setpoint	0	N
4	97	Vent X3, Cart	5	N
5	99	Backflush X3, Cart	20	N
6	206	Backflush X3, Flask	20	N
7	305	Set Reg Setpoint (10th psi)	38	N
8	310	Set Tolerance (100th psi)	20	N
9	257	Wait	40	N
10	308	Close Pressure Valve	1	N
11	257	Wait	15	N
12	307	Compare Pressures (10th psi)	38	N

Step	Function #	Function Name	Time (sec)	Global Time
13	317	Save Regulator Pressure	0	N
14	310	Set Tolerance (100th psi)	5	N
15	257	Wait	30	N
16	318	Compare Saved Pressure	0	N
17	97	Vent X3, Cart	40	N
18	310	Set Tolerance (100th psi)	10	N
19	307	Compare Pressures (10th psi)	0	N
20	309	Restore Reg Setpoint	0	N
21	98	Flush X3, Cart	5	N
22	94	Del X3, Waste	60	N
23	135	Flush Cart Reagent Block	10	N
24	143	Wash Cart Reagent Block	10	N
25	135	Flush Cart Reagent Block	30	N
26	207	Del X3, Waste, Flask	20	N
27	218	Flush Large Loop (Flask)	10	N
28	259	End	0	N

Regulator 9 Test Total run time: 1:35

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	303	Select Regulator	9	N
3	304	Save Regulator Setpoint	0	N
4	212	Bubble Flask	5	N
5	305	Set Reg Setpoint (10th psi)	38	N
6	310	Set Tolerance (100th psi)	20	N
7	257	Wait	25	N
8	308	Close Pressure Valve	1	N
9	257	Wait	15	N
10	307	Compare Pressures (10th psi)	38	N
11	317	Save Regulator Pressure	0	N
12	310	Set Tolerance (100th psi)	10	N
13	257	Wait	30	N
14	318	Compare Saved Pressure	0	N
15	212	Bubble Flask	15	N
16	310	Set Tolerance (100th psi)	10	N
17	307	Compare Pressures (10th psi)	0	N
18	309	Restore Reg Setpoint	0	N
19	212	Bubble Flask	5	N

Step	Function #	Function Name	Time (sec)	Global Time
20	259	End	0	N

Regulator 10 Test Total run time: 1:55

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	303	Select Regulator	10	N
3	304	Save Regulator Setpoint	0	N
4	223	Inject Position	0	N
5	257	Wait	5	N
6	221	Flush Injector	10	N
7	305	Set Reg Setpoint (10th psi)	38	N
8	310	Set Tolerance (100th psi)	20	N
9	257	Wait	25	N
10	308	Close Pressure Valve	1	N
11	257	Wait	15	N
12	307	Compare Pressures (10th psi)	38	N
13	317	Save Regulator Pressure	0	N
14	310	Set Tolerance (100th psi)	10	N
15	257	Wait	30	N
16	318	Compare Saved Pressure	0	N
17	285	Injector Sim Load	20	N
18	310	Set Tolerance (100th psi)	10	N
19	307	Compare Pressures (10th psi)	0	N
20	309	Restore Reg Setpoint	0	N
21	285	Injector Sim Load	10	N
22	259	End	0	N

Cart Reagent Blk Test Total run time: 1:20

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	303	Select Regulator	5	N
3	304	Save Regulator Setpoint	0	N
4	131	Dry Cart (top)	5	N
5	305	Set Reg Setpoint (10th psi)	38	N
6	310	Set Tolerance (100th psi)	20	N
7	284	Open Valves 11,15	15	N
8	308	Close Pressure Valve	1	N

Step	Function #	Function Name	Time (sec)	Global Time
9	284	Open Valves 11,15	10	N
10	307	Compare Pressures (10th psi)	38	N
11	317	Save Regulator Pressure	0	N
12	310	Set Tolerance (100th psi)	5	N
13	284	Open Valves 11,15	30	N
14	318	Compare Saved Pressure	0	N
15	345	Open Valves 1,11,15	10	N
16	310	Set Tolerance (100th psi)	10	N
17	307	Compare Pressures (10th psi)	0	N
18	309	Restore Reg Setpoint	0	N
19	344	Open Valves 7,11,15,16	10	N
20	259	End	0	N

Cart Input Blk Test Total run time: 1:20

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	303	Select Regulator	5	N
3	304	Save Regulator Setpoint	0	N
4	131	Dry Cart (top)	5	N
5	305	Set Reg Setpoint (10th psi)	38	N
6	310	Set Tolerance (100th psi)	20	N
7	293	Open Valves 15,23	15	N
8	308	Close Pressure Valve	1	N
9	293	Open Valves 15,23	10	N
10	307	Compare Pressures (10th psi)	38	N
11	317	Save Regulator Pressure	0	N
12	310	Set Tolerance (100th psi)	5	N
13	293	Open Valves 15,23	30	N
14	318	Compare Saved Pressure	0	N
15	344	Open Valves 7,11,15,16	10	N
16	310	Set Tolerance (100th psi)	10	N
17	307	Compare Pressures (10th psi)	0	N
18	309	Restore Reg Setpoint	0	N
19	344	Open Valves 7,11,15,16	10	N
20	259	End	0	N

Flask Input Test

Total run time: 1:05

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	303	Select Regulator	8	N
3	304	Save Regulator Setpoint	0	N
4	305	Set Reg Setpoint (10th psi)	38	N
5	310	Set Tolerance (100th psi)	20	N
6	294	Open Valve 24	5	N
7	308	Close Pressure Valve	1	N
8	294	Open Valve 24	10	N
9	307	Compare Pressures (10th psi)	38	N
10	317	Save Regulator Pressure	0	N
11	294	Open Valve 24	30	N
12	310	Set Tolerance (100th psi)	10	N
13	318	Compare Saved Pressure	0	N
14	218	Flush Large Loop (Flask)	10	N
15	310	Set Tolerance (100th psi)	10	N
16	307	Compare Pressures (10th psi)	0	N
17	309	Restore Reg Setpoint	0	N
18	218	Flush Large Loop (Flask)	10	N
19	259	End	0	N

Flask Leak Test

Total run time: 2:00

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	303	Select Regulator	8	N
3	304	Save Regulator Setpoint	0	N
4	305	Set Reg Setpoint (10th psi)	38	N
5	310	Set Tolerance (100th psi)	20	N
6	295	Open Valves 24,32	60	N
7	308	Close Pressure Valve	1	N
8	295	Open Valves 24,32	10	N
9	307	Compare Pressures (10th psi)	38	N
10	317	Save Regulator Pressure	0	N
11	295	Open Valves 24,32	30	N
12	310	Set Tolerance (100th psi)	5	N
13	318	Compare Saved Pressure	0	N
14	296	Open Valves 24,32,45	10	N
15	310	Set Tolerance (100th psi)	10	N

Step	Function #	Function Name	Time (sec)	Global Time
16	307	Compare Pressures (10th psi)	0	N
17	309	Restore Reg Setpoint	0	N
18	296	Open Valves 24,32,45	10	N
19	259	End	0	N

Waste System Test Total run time: 4:30

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	303	Select Regulator	7	N
3	304	Save Regulator Setpoint	0	N
4	299	Open Valves 49,57,59	120	N
5	310	Set Tolerance (100th psi)	15	N
6	308	Close Pressure Valve	1	N
7	77	Vent X1	30	N
8	307	Compare Pressures (10th psi)	25	N
9	317	Save Regulator Pressure	0	N
10	77	Vent X1	120	N
11	310	Set Tolerance (100th psi)	10	N
12	318	Compare Saved Pressure	0	N
13	309	Restore Reg Setpoint	0	N
14	259	End	0	N

Shutdown Procedure List

Short-Term Shutdown

Total run time: 21:35

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	145	Wash Small Loop (Cart)	20	N
3	146	Wash Large Loop (Cart)	20	N
4	143	Wash Cart Reagent Block	20	N
5	144	Wash Cart Solvent Block	20	N
6	106	Wash Input Block (S2)	15	N
7	111	Wash Input Block (S3)	15	N
8	107	Wash Output Block (S2)	15	N
9	112	Wash Output Block (S3)	15	N
10	139	Flush Small Loop (Cart)	60	N
11	140	Flush Large Loop (Cart)	60	N
12	135	Flush Cart Reagent Block	60	N
13	137	Flush Input Block	60	N
14	138	Flush Output Block	60	N
15	136	Flush Cart Solvent Block	60	N
16	9	Backflush R1	20	N
17	19	Backflush R2g	20	N
18	29	Backflush R3	60	N
19	39	Backflush R3g	20	N
20	49	Backflush S1	20	N
21	59	Backflush S2	20	N
22	69	Backflush S3	20	N
23	79	Backflush X1	60	N
24	89	Backflush X1g	20	N
25	99	Backflush X3, Cart	60	N
26	219	Wash Small Loop (Flask)	15	N
27	217	Flush Small Loop (Flask)	60	N
28	220	Wash Large Loop (Flask)	15	N
29	218	Flush Large Loop (Flask)	60	N
30	171	Del S4, Flask	15	N
31	213	Dry Flask	10	N
32	215	Empty Flask	20	N
33	171	Del S4, Flask	10	N
34	213	Dry Flask	10	N
35	222	Flush Flask/Injector	60	N
36	156	Backflush R4	20	N
37	166	Backflush R5	20	N

Step	Function #	Function Name	Time (sec)	Global Time
38	176	Backflush S4	20	N
39	186	Backflush X2	60	N
40	196	Backflush X2g	20	N
41	206	Backflush X3, Flask	60	N
42	259	End	0	N

Post-Run Valve Block Wash - X3

Total run time: 12:56

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	303	Select Regulator	8	N
3	304	Save Regulator Setpoint	0	N
4	305	Set Reg Setpoint (10th psi)	50	N
5	215	Empty Flask	5	N
6	201	Del X3, Flask	30	N
7	94	Del X3, Waste	20	N
8	116	Wash Input Block (X3)	60	N
9	117	Wash Output Block (X3)	60	N
10	257	Wait	120	N
11	136	Flush Cart Solvent Block	40	N
12	140	Flush Large Loop (Cart)	30	N
13	135	Flush Cart Reagent Block	40	N
14	137	Flush Input Block	40	N
15	141	Flush Transfer Line	40	N
16	212	Bubble Flask	5	N
17	213	Dry Flask	5	N
18	215	Empty Flask	20	N
19	201	Del X3, Flask	30	N
20	217	Flush Small Loop (Flask)	20	N
21	218	Flush Large Loop (Flask)	20	N
22	213	Dry Flask	20	N
23	226	Load Position	1	N
24	285	Injector Sim Load	40	N
25	222	Flush Flask/Injector	80	N
26	171	Del S4, Flask	15	N
27	213	Dry Flask	10	N
28	215	Empty Flask	20	N
29	309	Restore Reg Setpoint	0	N
30	205	Flush X3, Flask	5	N
31	259	End	0	N

Startup Procedure

Startup Total run time: 14:15

Step	Function #	Function Name	Time (sec)	Global Time
1	258	Begin	0	N
2	8	Flush R1	10	N
3	4	Del R1, Waste	15	N
4	135	Flush Cart Reagent Block	10	N
5	78	Flush X1	15	N
6	74	Del X1, Waste	60	N
7	135	Flush Cart Reagent Block	10	N
8	18	Flush R2g	10	N
9	14	Del R2g, Waste	10	N
10	58	Flush S2	15	N
11	54	Del S2, Waste	15	N
12	136	Flush Cart Solvent Block	20	N
13	143	Wash Cart Reagent Block	15	N
14	135	Flush Cart Reagent Block	20	N
15	303	Select Regulator	3	N
16	304	Save Regulator Setpoint	0	N
17	305	Set Reg Setpoint (10th psi)	15	N
18	28	Flush R3	10	N
19	24	Del R3, Waste	60	N
20	34	Del R3g, Waste	10	N
21	309	Restore Reg Setpoint	0	N
22	136	Flush Cart Solvent Block	10	N
23	144	Wash Cart Solvent Block	15	N
24	136	Flush Cart Solvent Block	20	N
25	68	Flush S3	15	N
26	64	Del S3, Waste	15	N
27	136	Flush Cart Solvent Block	10	N
28	48	Flush S1	15	N
29	44	Del S1, Waste	10	N
30	136	Flush Cart Solvent Block	10	N
31	144	Wash Cart Solvent Block	15	N
32	106	Wash Input Block (S2)	15	N
33	111	Wash Input Block (S3)	15	N
34	107	Wash Output Block (S2)	15	N
35	112	Wash Output Block (S3)	15	N
36	137	Flush Input Block	40	N
37	138	Flush Output Block	40	N

Step	Function #	Function Name	Time (sec)	Global Time
38	136	Flush Cart Solvent Block	40	N
39	155	Flush R4	10	N
40	157	Del R4, Waste	30	N
41	218	Flush Large Loop (Flask)	10	N
42	165	Flush R5	5	N
43	167	Del R5, Waste	10	N
44	218	Flush Large Loop (Flask)	10	N
45	175	Flush S4	15	N
46	177	Del S4, Waste	15	N
47	218	Flush Large Loop (Flask)	20	N
48	171	Del S4, Flask	15	N
49	213	Dry Flask	10	N
50	215	Empty Flask	20	N
51	171	Del S4, Flask	15	N
52	213	Dry Flask	10	N
53	215	Empty Flask	40	N
54	259	End	0	N

Getting Help

D

Technical Support

Contacting Technical Support

You can contact Applied Biosystems for technical support by telephone or fax, by e-mail, or through the Internet. You can order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents 24 hours a day. In addition, you can download documents in PDF format from the Applied Biosystems Web site (please see the section "To Obtain Documents on Demand" following the telephone information below).

To Contact Technical Support by E-Mail

Contact technical support by e-mail for help in the following product areas:

Product Area	E-mail address
Genetic Analysis (DNA Sequencing)	galab@appliedbiosystems.com
Sequence Detection Systems and PCR	pcrlab@appliedbiosystems.com
Protein Sequencing, Peptide and DNA Synthesis	corelab@appliedbiosystems.com
Biochromatography, PerSeptive DNA, PNA and Peptide Synthesis systems, CytoFluor®, FMAT™, Voyager™, and Mariner™ Mass Spectrometers	tsupport@appliedbiosystems.com
LC/MS (Applied Biosystems/MDS Sciex)	apisupport@sciex.com or api3-support@sciex.com
Chemiluminescence (Tropix)	tropix@appliedbiosystems.com

Hours for Telephone Technical Support

In the United States and Canada, technical support is available at the following times:

Product	Hours
Chemiluminescence	8:30 a.m. to 5:30 p.m. Eastern Time
Framingham support	8:00 a.m. to 6:00 p.m. Eastern Time
All Other Products	5:30 a.m. to 5:00 p.m. Pacific Time

To Contact Technical Support by Telephone or Fax

In North America

To contact Applied Biosystems Technical Support, use the telephone or fax numbers given below. (To open a service call for other support needs, or in case of an emergency, dial **1-800-831-6844** and press **1**.)

Product or Product Area	Telephone Dial...	Fax Dial...
ABI PRISM® 3700 DNA Analyzer	**1-800-831-6844**, then press **8**	**1-650-638-5981**
DNA Synthesis	**1-800-831-6844**, then press **21**	**1-650-638-5981**
Fluorescent DNA Sequencing	**1-800-831-6844**, then press **22**	**1-650-638-5981**
Fluorescent Fragment Analysis (includes GeneScan® applications)	**1-800-831-6844**, then press **23**	**1-650-638-5981**
Integrated Thermal Cyclers (ABI PRISM® 877 and Catalyst 800 instruments)	**1-800-831-6844**, then press **24**	**1-650-638-5981**
ABI PRISM® 3100 Genetic Analyzer	**1-800-831-6844**, then press **26**	**1-650-638-5981**
BioInformatics (includes BioLIMS®, BioMerge™, and SQL GT™ applications)	**1-800-831-6844**, then press **25**	**1-505-982-7690**
Peptide Synthesis (433 and 43X Systems)	**1-800-831-6844**, then press **31**	**1-650-638-5981**
Protein Sequencing (Procise® Protein Sequencing Systems)	**1-800-831-6844**, then press **32**	**1-650-638-5981**
PCR and Sequence Detection	**1-800-762-4001**, then press **1** for PCR, **2** for the 7700 or 5700, **6** for the 6700 or dial **1-800-831-6844**, then press **5**	**1-240-453-4613**
Voyager™ MALDI-TOF Biospectrometry and Mariner™ ESI-TOF Mass Spectrometry Workstations	**1-800-899-5858**, then press **13**	**1-508-383-7855**
Biochromatography (BioCAD® Workstations and Poros® Perfusion Chromatography Products)	**1-800-899-5858,** then press **14**	**1-508-383-7855**
Expedite™ Nucleic acid Synthesis Systems	**1-800-899-5858**, then press **15**	**1-508-383-7855**
Peptide Synthesis (Pioneer™ and 9050 Plus Peptide Synthesizers)	**1-800-899-5858**, then press **15**	**1-508-383-7855**
PNA Custom and Synthesis	**1-800-899-5858**, then press **15**	**1-508-383-7855**
FMAT™ 8100 HTS System and Cytofluor® 4000 Fluorescence Plate Reader	**1-800-899-5858**, then press **16**	**1-508-383-7855**
Chemiluminescence (Tropix)	**1-800-542-2369** (U.S. only), or **1-781-271-0045**	**1-781-275-8581**
Applied Biosystems/MDS Sciex	**1-800-952-4716**	**1-650-638-6223**

Outside North America

Region	Telephone Dial...	Fax Dial...
Africa and the Middle East		
Africa (English Speaking) and West Asia (Fairlands, South Africa)	**27 11 478 0411**	**27 11 478 0349**
South Africa (Johannesburg)	**27 11 478 0411**	**27 11 478 0349**
Middle Eastern Countries and North Africa (Monza, Italia)	**39 (0)39 8389 481**	**39 (0)39 8389 493**
Eastern Asia, China, Oceania		
Australia (Scoresby, Victoria)	**61 3 9730 8600**	**61 3 9730 8799**
China (Beijing)	**86 10 64106608**	**86 10 64106617**
Hong Kong	**852 2756 6928**	**852 2756 6968**
Korea (Seoul)	**82 2 593 6470/6471**	**82 2 593 6472**
Malaysia (Petaling Jaya)	**60 3 758 8268**	**60 3 754 9043**
Singapore	**65 896 2168**	**65 896 2147**
Taiwan (Taipei Hsien)	**886 2 22358 2838**	**886 2 2358 2839**
Thailand (Bangkok)	**66 2 719 6405**	**66 2 319 9788**
Europe		
Austria (Wien)	**43 (0)1 867 35 75 0**	**43 (0)1 867 35 75 11**
Belgium	**32 (0)2 712 5555**	**32 (0)2 712 5516**
Czech Republic and Slovakia (Praha)	**420 2 61 222 164**	**420 2 61 222 168**
Denmark (Naerum)	**45 45 58 60 00**	**45 45 58 60 01**
Finland (Espoo)	**358 (0)9 251 24 250**	**358 (0)9 251 24 243**
France (Paris)	**33 (0)1 69 59 85 85**	**33 (0)1 69 59 85 00**
Germany (Weiterstadt)	**49 (0) 6150 101 0**	**49 (0) 6150 101 101**
Hungary (Budapest)	**36 (0)1 270 8398**	**36 (0)1 270 8288**
Italy (Milano)	**39 (0)39 83891**	**39 (0)39 838 9492**
Norway (Oslo)	**47 23 12 06 05**	**47 23 12 05 75**
Poland, Lithuania, Latvia, and Estonia (Warszawa)	**48 (22) 866 40 10**	**48 (22) 866 40 20**
Portugal (Lisboa)	**351 (0)22 605 33 14**	**351 (0)22 605 33 15**
Russia (Moskva)	**7 095 935 8888**	**7 095 564 8787**
South East Europe (Zagreb, Croatia)	**385 1 34 91 927**	**385 1 34 91 840**
Spain (Tres Cantos)	**34 (0)91 806 1210**	**34 (0)91 806 1206**
Sweden (Stockholm)	**46 (0)8 619 4400**	**46 (0)8 619 4401**
Switzerland (Rotkreuz)	**41 (0)41 799 7777**	**41 (0)41 790 0676**
The Netherlands (Nieuwerkerk a/d IJssel)	**31 (0)180 331400**	**31 (0)180 331409**
United Kingdom (Warrington, Cheshire)	**44 (0)1925 825650**	**44 (0)1925 282502**
All other countries not listed (Warrington, UK)	**44 (0)1925 282481**	**44 (0)1925 282509**
Japan		
Japan (Hacchobori, Chuo-Ku, Tokyo)	**81 3 5566 6230**	**81 3 5566 6507**

Region	Telephone Dial...	Fax Dial...
Latin America		
Del.A. Obregon, Mexico	**305-670-4350**	**305-670-4349**

To Reach Technical Support Through the Internet

We strongly encourage you to visit our Web site for answers to frequently asked questions and for more information about our products. You can also order technical documents or an index of available documents and have them faxed or e-mailed to you through our site. The Applied Biosystems Web site address is

http://www.appliedbiosystems.com/techsupp

To submit technical questions from North America or Europe:

Step	Action
1	Access the Applied Biosystems Technical Support Web site.
2	Under the **Troubleshooting** heading, click **Support Request Forms,** then select the relevant support region for the product area of interest.
3	Enter the requested information and your question in the displayed form, then click **Ask Us RIGHT NOW** (blue button with yellow text).
4	Enter the required information in the next form (if you have not already done so), then click **Ask Us RIGHT NOW**. You will receive an e-mail reply to your question from one of our technical experts within 24 to 48 hours.

To Obtain Documents on Demand

Free, 24-hour access to Applied Biosystems technical documents, including MSDSs, is available by fax or e-mail or by download from our Web site.

To order documents...	Then...
by index number	a. Access the Applied Biosystems Technical Support Web site at **http://www.appliedbiosystems.com/techsupp** b. Click the **Index** link for the document type you want, then find the document you want and record the index number. c. Use the index number when requesting documents following the procedures below.
by phone for fax delivery	a. From the U.S. or Canada, call **1-800-487-6809,** or from outside the U.S. and Canada, call **1-858-712-0317**. b. Follow the voice instructions to order the documents you want. **Note** There is a limit of five documents per request.
through the Internet for fax or e-mail delivery	a. Access the Applied Biosystems Technical Support Web site at **http://www.appliedbiosystems.com/techsupp** b. Under **Resource Libraries**, click the type of document you want. c. Enter or select the requested information in the displayed form, then click **Search**. d. In the displayed search results, select a check box for the method of delivery for each document that matches your criteria, then click **Deliver Selected Documents Now** (or click the PDF icon for the document to download it immediately). e. Fill in the information form (if you have not previously done so), then click **Deliver Selected Documents Now** to submit your order. **Note** There is a limit of five documents per request for fax delivery but no limit on the number of documents you can order for e-mail delivery.

Warranty

E

Applied Biosystems Limited Warranty Statement

Applied Biosystems warrants to the customer that, for a period ending on the earlier of one year(s) from the completion of installation or (15) month(s) from the date of shipment to the customer (the “Warranty Period”), the Procise®, purchased by the customer (the “Instrument”) will be free from defects in material and workmanship, and will perform in accordance with the minimum specifications (set forth in/set out in/contained in) the Instrument User’s Manual and/or the Instrument’s Product Specification Sheet (the “Specifications”).

During the Warranty Period, if the Instrument’s hardware becomes damaged or contaminated or if the Instrument otherwise fails to meet the Specifications, Applied Biosystems will repair or replace the Instrument so that it meets the Specifications, at Applied Biosystems expense. However, if the valves or reagent lines become damaged or contaminated, or if the chemical performance of the Instrument otherwise deteriorates due to solvents and/or reagents other than those supplied or expressly recommended by Applied Biosystems, Applied Biosystems will return the Instrument to Specification at the customer’s request and at the customer’s expense. After this service is performed, coverage of the parts repaired or replaced will be restored thereafter for the remainder of the original Warranty Period.

This Warranty does not extend to any Instrument or part which has been (a) the subject of an accident, misuse, or neglect, (b) modified or repaired by a party other than Applied Biosystems, or (c) used in a manner not in accordance with the instructions contained in the Instrument User’s Manual. This Warranty does not cover the customer-installable accessories or customer-installable consumable parts for the Instrument that are listed in the Instrument User’s Manual. Those items are covered by their own warranties. Applied Biosystems obligation under this Warranty is limited to repairs or replacements that Applied Biosystems deems necessary to correct those failures of the Instrument to meet the Specifications of which Applied Biosystems is notified prior to expiration of the Warranty Period. All repairs and replacements under this Warranty will be performed by Applied Biosystems on site at the Customer’s location at Applied Biosystems sole expense.

No agent, employee, or representative of Applied Biosystems has any authority to bind Applied Biosystems to any affirmation, representation, or warranty concerning the Instrument that is not contained in Applied Biosystems printed product literature or this Warranty Statement. Any such affirmation, representation or warranty made by any agent, employee, or representative of Applied Biosystems will not be binding on Applied Biosystems.

Applied Biosystems shall not be liable for any incidental, special, or consequential loss, damage or expense directly or indirectly arising from the purchase or use of the Instrument. Applied Biosystems makes no warranty whatsoever with regard to products or parts furnished by third parties. This Warranty is limited to the original location of installation and electrical power connection and is not transferable.

THIS WARRANTY IS THE SOLE AND EXCLUSIVE WARRANTY AS TO THE INSTRUMENT AND IS IN LIEU OF ANY OTHER EXPRESS OR IMPLIED WARRANTIES, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE AND IS IN LIEU OF ANY OTHER OBLIGATION ON THE PART OF APPLIED BIOSYSTEMS.

Index

Headquarters
850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free: +1 800.345.5224
Fax: +1 650.638.5884

Worldwide Sales Offices

Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches into 150 countries on six continents. For international office locations, please call our local office or refer to our web site at www.appliedbiosystems.com.

www.appliedbiosystems.com

Applied Biosystems

Applera Corporation is committed to providing the world's leading technology and information for life scientists. Applera Corporation consists of the Applied Biosystems and Celera Genomics businesses.

Printed in the USA, 05/2002
Part Number 4314375B

an Applera business