

Ettan™ DALT*six* and Ettan DALT*twelve* Systems

Operating Instructions

Original instructions

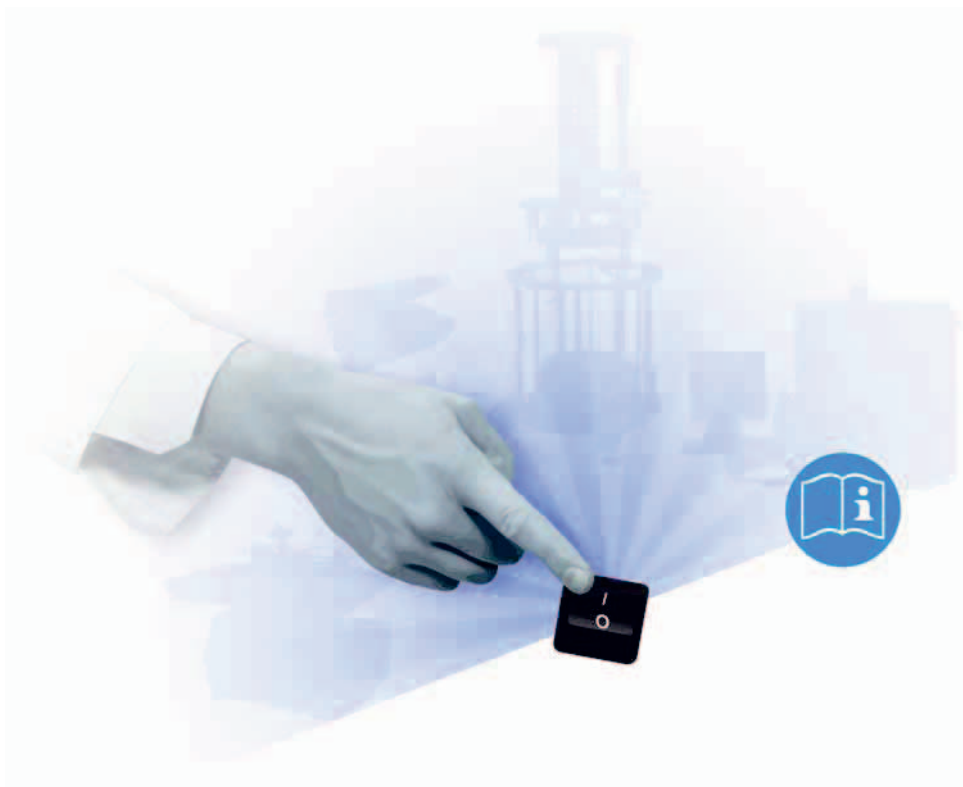


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1 Introduction

Purpose of the Operating Instructions

The Operating Instructions provide you with the instructions needed to handle Ettan DALTsix and Ettan DALTwelve Systems in a safe way.

Prerequisites

In order to operate Ettan DALTsix and Ettan DALTwelve Systems safely and according to the intended purpose the following prerequisites must be met:

- You should be acquainted with the use of general laboratory equipment and with handling of biological materials.
- You should understand the concepts of electrophoresis.
- You must read the Safety Instructions in *Chapter 2* of these Operating Instructions.
- The system should be installed according to the instructions in *Chapter 3* of these Operating Instructions.

In this chapter

This chapter contains important user information and a general description of Ettan DALTsix and Ettan DALTwelve Systems and their intended use.

1.1 Important user information

Read this before using Ettan DALTsix and Ettan DALTwelve Systems



All users must read the Safety Instructions in *Chapter 2* of these Operating Instructions before installing, using or maintaining the instrument.

Do not operate Ettan DALTsix and Ettan DALTwelve Systems in any other way than described in the user documentation. If you do, you may be exposed to hazards that can lead to personal injury and you may cause damage to the equipment.

Intended use

The Ettan DALTsix and Ettan DALTtwelve Systems are designed to handle multiple large second dimension electrophoresis gels in a simple, efficient, and reproducible manner.

Ettan DALTsix and Ettan DALTtwelve Systems are intended for research use only, and shall not be used in any clinical procedures, or for diagnostic purposes.

Safety notices

These Operating Instructions contain WARNINGS, CAUTIONS and NOTICES concerning the use of the product, with meanings as defined below.



WARNING

WARNING indicates a hazardous situation which, if not avoided, could result in death or serious injury. It is important not to proceed until all stated conditions are met and clearly understood.



CAUTION

CAUTION indicates a hazardous situation which, if not avoided, could result in minor or moderate injury. It is important not to proceed until all stated conditions are met and clearly understood.



NOTICE

NOTICE indicates instructions that must be followed to avoid damage to the product or other equipment.

Notes and tips

Note: A Note is used to indicate information that is important for trouble-free and optimal use of the product.

Tip: A tip contains useful information that can improve or optimize your procedures.

Typographical conventions

Software texts and commands are identified by ***bold italic*** text. A colon is used to separate menu levels (e.g. ***File:Open*** refers to the ***Open*** option in the ***File*** menu).

1.2 Regulatory information

This section lists the directives and standards that are fulfilled by Ettan DALTsix and Ettan DALTwelve Systems.

Manufacturing information

Requirement	Content
Name and address of manufacturer	GE Healthcare Bio-Sciences AB, Björkgatan 30, SE 751 84 Uppsala Sweden
Name and ID of notified body	INTERTEK SEMKO AB, NB 0413
Place and date of declaration	Uppsala, Sweden, May 2009
Identity of person authorized to sign DoC	See EC Declaration of Conformity

CE Conformity

Directive	Title
2006/42/EC	Machinery Directive (MD)
2006/95/EC	Low Voltage Directive (LVD)
2004/108/EC	ElectroMagnetic Compatibility (EMC) Directive

International standards

Standard	Description	Notes
EN 61010-1, IEC 61010-1, CAN/CSA-C22.2 No. 61010-1	Safety requirements for electrical equipment for measurement, control and laboratory use	
EN 61326-1	EMC emissions and immunity requirements for electrical equipment measurement, control and laboratory use	Harmonized with 2004/108/EC
EN-ISO 12100-1, 12100-2	Safety of machinery – Basic concepts, general principles and design	Harmonized with 2006/42/EC
EN-ISO 14121-1, 14121-2	Safety of machinery – Principles of risk assessment	Harmonized with 2006/42/EC

CE marking



The CE marking and the corresponding Declaration of Conformity is valid for the instrument when it is:

- used as a stand-alone unit, or
- connected to other CE-marked instruments, or
- connected to other products recommended or described in the user documentation, and
- used in the same state as it was delivered from GE Healthcare, except for alterations described in the user documentation or explicitly authorized by GE Healthcare.

Regulatory compliance of connected equipment

Any equipment connected to Ettan DALTsix and Ettan DALTwelve Systems should meet the safety requirements of EN 61010-1/IEC61010-1 or relevant harmonized standards. Within the European Union, connected equipment must be CE-marked.

1.3 Instrument

This chapter contains a general description of Ettan DALTsix and Ettan DALTtwelve Systems.

General description

In 2-D electrophoresis, proteins are separated according to isoelectric point by isoelectric focusing, most reliably on immobilized pH gradient (IPG) strips using the IPGphor™ IEF System. The second-dimension electrophoresis separates the proteins on the basis of their molecular mass using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The Ettan DALTsix and Ettan DALTtwelve Systems are designed to handle multiple large second dimension gels in a simple, efficient, and reproducible manner.

A sample, focused in an IPG strip, is placed on the cathodic (upper) surface of a slab gel and sealed in place with agarose. The Gel Cassette is then inserted into the electrophoresis unit through the buffer seal slots flanked by rubber gaskets. Any unused slots are filled with the blank cassette inserts. The buffer seal is an effective current and liquid barrier. For more information, see *Chapter 4*.

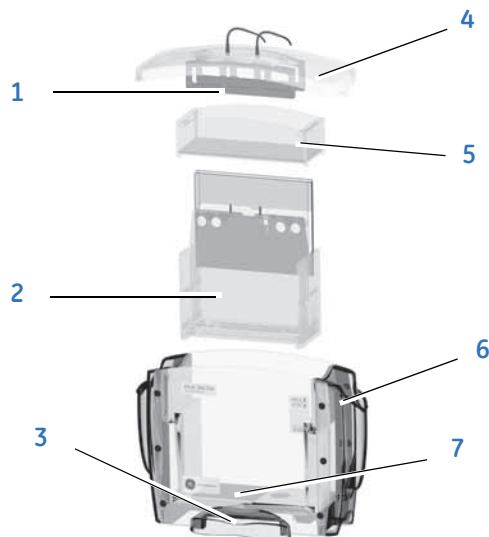
System components

The following components are included in Ettan DALTsix and Ettan DALTtwelve Systems:

- Electrophoresis unit
- Power Supply/Control Unit
- Gel Caster
- Gel Casting Cassettes
- Gradient maker

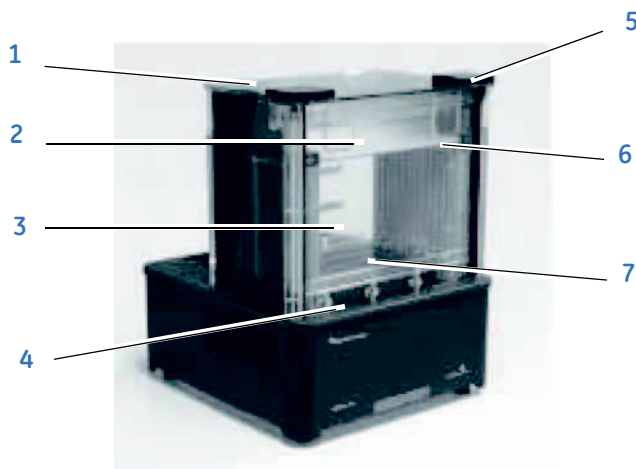
The Electrophoresis units

Description of Ettan DALTsix Electrophoresis unit



Part	Description	Part	Description
1	Safety interlock	5	Upper buffer chamber
2	Anode assembly\cassette carrier	6	Lower buffer chamber
3	Buffer circulation pump	7	Ceramic heat exchanger
4	Lid with leads		

Description of Ettan DALTtwelve Electrophoresis unit



Part	Description	Part	Description
1	Lid with leads (cathode)	5	Safety interlock
2	Upper buffer chamber	6	Rubber gasket
3	Lower buffer chamber	7	Ceramic heat exchanger
4	Buffer circulation pump		

Electrophoresis unit data

The Ettan DALTsix and Ettan DALTtwelve can accommodate a different number of gels, either 1 mm or 1.5 mm thick, in a common tank under identical conditions.

Electrophoresis system	Gel size	Gel capacity
Ettan DALTsix	25.5 × 20.5 cm	6
Ettan DALTtwelve	25.5 × 20.5 cm	12

Power supply/control unit

Electrophoresis system	Power supply unit	Requirements
Ettan DALTsix	EPS 601 Power Supply or any other power supply with similar specifications	600 V, 400 mA, or 100 W
Ettan DALT <i>twelve</i>	Ettan DALT <i>twelve</i> Power Supply/Control Unit	600 V, 1 A, or 200 W

Pre-cast Gels and Gel cassettes

Pre-cast gels

If fluorescent staining/labeling techniques will be used, do not run gels cast on plastic backing, as it can pose a problem of high background with some dyes during subsequent analysis. Instead, a DIGE Gel can be used. A DIGE Gel is a 12.5% precast polyacrylamide gel in a low fluorescent glass cassette for second dimension 2-D electrophoresis and specially developed for 2-D DIGE analysis. It is intended to be used in Ettan DALTsix or Ettan DALT*twelve* systems together with the DIGE Buffer Kit. For more information on DIGE Gels, see *Ettan DIGE System User Manual*.

DALT Gel 12.5 is a precast polyacrylamide gel (25.5 × 19.6 cm, 1 mm thick) for the second dimension of 2-D electrophoresis. The gel is provided already cast onto a plastic support film. The gel is a homogeneous 12.5% polyacrylamide gel. It is intended to be used in Ettan DALTsix or Ettan DALT*twelve* systems together with the DALT Buffer Kit, see *2-D Electrophoresis Principles and Methods*.

DALT Pre-cast Gel Cassettes

The DALT Gel 12.5 pre-cast gels are inserted into a specially designed reusable cassette (see below) and run vertically in the Ettan DALTsix and Ettan DALT*twelve* Systems.



Gel casting equipment

Gel Caster

The Ettan DALTsix and Ettan DALTtwelve Gel Casters can hold a different number of gel cassettes with separator sheets, for casting homogenous or gradient gels.

System	Cassette capacity, 1.0 mm gels	Cassette capacity, 1.5 mm gels
Ettan DALTsix Gel Caster 	6	6
Ettan DALTtwelve Gel Caster 	14	13

Fewer gels can be cast at one time by using blank cassette inserts to occupy unneeded volume. For more information on gel casting, see Ettan DALTsix and Ettan DALTtwelve *User Manuals*.

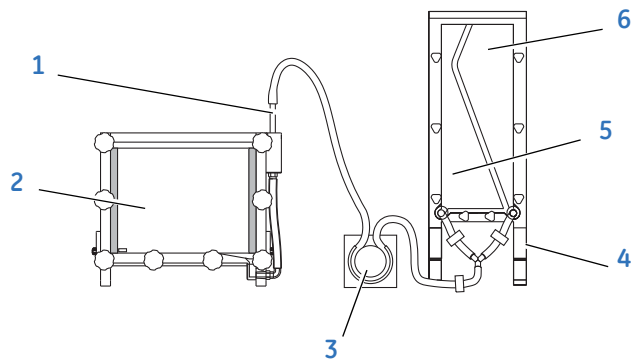
Gel Casting Cassettes

The Ettan DALTsix and Ettan DALTtwelve use the same cassettes. The gel casting cassettes are pre assembled. Two glass plates are joined along one edge by a hinge strip of silicone rubber, and the vinyl side spacers (1.0 mm or 1.5 mm thick) are glued in place. To complete assembly, close the two plates like a book and press the plates together over the length of the spacer. Gels are removed by opening the book after the run and carefully lifting out the gel slab.



Gradient maker

A Gradient maker is used to make gradient gels using two gel solutions of different acrylamide concentrations and densities — a light solution and a heavy solution. Below is a description of Ettan DALTtwelve Gradient maker.



Part	Description	Part	Description
1	Feed tube	4	Gradient maker
2	Gel caster	5	Heavy chamber
3	Pump	6	Light chamber

For more information on how to cast gradient gels, see Ettan DALTsix and Ettan DALTtwelve *User Manuals*. For information on how to use the pump, refer to the user documentation supplied with the pump.

2 Safety instructions

This chapter describes safety compliance, safety labels, general safety precautions, emergency procedures, power failure and recycling of Ettan DALTsix and Ettan DALTwelve Systems.

2.1 Safety precautions

Introduction

The Ettan DALTsix and Ettan DALTwelve Systems are powered by mains voltage and handles liquids that may be hazardous. Before installing, operating or maintaining the system, you must be aware of the hazards described in this manual. Follow the instructions provided to avoid personal injury or damage to the equipment.

The safety precautions in this section are grouped into the following categories:

- General precautions
- Using flammable liquids
- Personal protection
- Installing and moving the instrument
- System operation
- Maintenance

General precautions

**WARNING**

Do not operate the Ettan DALTsix and Ettan DALTwelve Systems in any other way than described in the Ettan DALTsix and Ettan DALTwelve Systems manuals.

**WARNING**

Operation and user maintenance of the Ettan DALTsix and Ettan DALTwelve Systems should be performed by properly trained personnel only.



WARNING

Do not use any accessories not supplied or recommended by GE Healthcare.



WARNING

Do not use the Ettan DALTsix and Ettan DALTtwelve Systems if they are not working properly, nor if it have suffered any damage, for example:

- damage to the power cord or its plug
- damage caused by dropping the equipment
- damage caused by splashing liquid onto items not supplied or recommended by GE Healthcare.



WARNING

Operation and user maintenance of the Ettan DALTsix and Ettan DALTtwelve Systems should be performed by properly trained personnel only.



WARNING

Ettan DALTsix and Ettan DALTtwelve Systems are high voltage instruments that can cause fatal electrical shock if the safety features are disabled. The safety lid must be securely closed before starting a protocol.



WARNING

The instrument covers must not be opened by the user. It contains electrical circuits which can give a lethal electric shock. Service and planned maintenance should be performed by personnel authorized by GE Healthcare.



WARNING

Disconnect all units from mains power supply before removing the safety lid, unloading the gels, draining and cleaning the electrophoresis unit.

Using flammable liquids

When using flammable liquids with the Ettan DALTsix and Ettan DALTtwelve Systems system, follow these precautions to avoid any risk of fire or explosion.



WARNING

Fire Hazard. Before starting the system, make sure that there is no leakage.



WARNING

A fume hood or similar ventilation system shall be installed when flammable or noxious substances are used.

Personal protection



WARNING

Always use appropriate personal protective equipment during operation and maintenance of Ettan DALTsix and Ettan DALTtwelve Systems.



WARNING

Hazardous substances. When using hazardous chemical and biological agents, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation and maintenance of the system's.



WARNING

Spread of biological agents. The operator has to take all necessary actions to avoid spreading hazardous biological agents in the vicinity of the instrument. The facility should comply with the national code of practice for biosafety.

Installing and moving the instrument



WARNING

Supply voltage. Make sure that the supply voltage at the wall outlet corresponds to the marking on the instrument, before connecting the power cord.



WARNING

Protective ground. The Ettan DALTsix and Ettan DALTtwelve Systems must always be connected to a grounded power outlet.



WARNING

Power cord. Only use power cords delivered or approved by GE Healthcare.



WARNING

Access to power switch and power cord. Do not block the rear and side panel of the instrument. The Power switch must always be easy to access. The power cord must always be easy to disconnect.



WARNING

Use EPS 601 as power supply for Ettan DALTsix.



WARNING

Only connect MultiTemp III, or a circulating water bath with earthed water pipe and low pressure water circulation. Do not connect the heat exchanger to a water tap or any other water source with unregulated pressure that may exceed 82 kPa (12 PSI).



WARNING

Be careful when lifting and moving the electrophoresis unit. It is best to move the unit when empty.

System operation



WARNING

Hazardous biological agents during run. When using hazardous biological agents, flush the entire system tubing with bacteriostatic solution followed by a neutral buffer and finally distilled water, before service and maintenance.



WARNING
Avoid spilling liquids on the body of the instrument. If large volumes of liquid have penetrated the casing of the instrument and come into contact with the electrical components, immediately switch off the instrument and contact an authorized service technician.

Maintenance



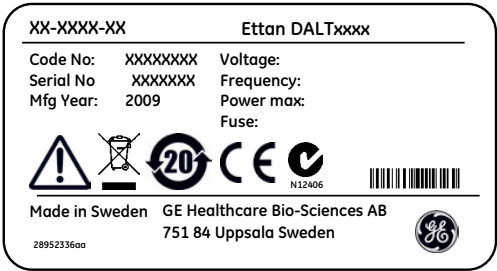
WARNING
Do not remove the main cover. There are no userserviceable components inside, and you can be exposed to high voltage.

2.2 Labels

This section describes the safety labels and labels concerning hazardous substances that are attached to the Ettan DALTsix and Ettan DALTwelve Systems. For information about marking of the computer equipment, refer to the manufacturer’s instructions.

Labels on the instrument

The illustration below shows an example of the identification label that is attached to the Ettan DALTsix and Ettan DALTwelve Systems.



Symbols used in safety labels

Label	Meaning
	Warning! Read the user documentation before using the system. Do not open any covers or replace parts unless specifically stated in the user documentation.
	Indicates that hazardous voltages are generated inside the instrument.
	The system complies with the requirements for electromagnetic compliance (EMC) in Australia and New Zealand.
	The system complies with applicable European directives.

Labels concerning hazardous substances

Label	Meaning
	This symbol indicates that the waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.

Label	Meaning
	This symbol indicates that the product contains hazardous materials in excess of the limits established by the Chinese standard SJ/T11363-2006 Requirements for Concentration Limits for Certain Hazardous Substances in Electronics.

2.3 Emergency procedures

This section describes how to do an emergency shutdown of Ettan DALTsix and Ettan DALTwelve. The section also describes the result in the event of power failure.

Emergency procedures

In an emergency situation, do as follows to stop the run:

Step	Action
1	Switch off power to the instrument by pressing the Power switch on the external power supply to the 0 position. If required, disconnect the mains power cord. The run is interrupted immediately.
2	For Ettan DALTsix: If required, disconnect the Ettan DALTsix mains power cord.

Power failure

The result of a power failure depends on which unit that is affected.

Power failure to...	will result in...
Ettan DALTsix and Ettan DALTwelve	The run is interrupted immediately, in an undefined state

2.4 Recycling information

The equipment shall be decontaminated before decommissioning and all local regulations shall be followed with regard to scrapping of the equipment.

Disposal, general instructions

When taking Ettan DALTsix and Ettan DALTwelve out of service, the different materials must be separated and recycled according to national and local environmental regulations.

Recycling of hazardous substances

Ettan DALTsix and Ettan DALTwelve contain hazardous substances. Detailed information is available from your GE Healthcare representative.

Disposal of electrical components

Waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.



3 Installation

Ettan DALTsix and Ettan DALTwelve are delivered in protective packing material and shall be unpacked with great care.

Any equipment connected to Ettan DALTsix and Ettan DALTwelve must fulfill applicable standards and local regulations.

For detailed information on Installation, see Ettan DALTsix and Ettan DALTwelve *User Manuals*.

3.1 Site requirements

Parameter	Requirement
Electrical power	220-240 V, 50-60 Hz
Ambient temperature	4°C to 40°C
Placement	Stable laboratory bench
Humidity	0 to 90%, non-condensing

3.2 Unpacking

- Check the equipment for damage before starting assembly and installation.
- Document any damage and contact your local GE Healthcare representative.

Remove straps and packing material and stand equipment upright before starting installation.

3.3 Placement

Position the electrophoresis unit close to a sink for easy rinsing and draining. It must be placed on a stable support such as a bench or sturdy table.

A length of rubber or vinyl tubing sufficiently long to reach a sink should be attached to the drain port on the back of the unit before use. The unit should not be placed on bench paper or any other material that might be pulled in by the air intake fans, as any hindrance to air circulation will reduce the cooling capacity.

3 Installation

3.4 Transport

For Ettan DALTsix: The tubing leading to and from the heat exchanger should be connected to a circulating water bath such as the MultiTemp III; the heat exchanger should not be connected to a water tap or any other coolant supply that lacks pressure regulation.

The Power Supply should be placed conveniently near the electrophoresis unit in a dry place without risk for liquid exposure

3.4 Transport

Before moving the system:

- remove liquids and gels from the separation unit
- disconnect all cables
- lift the instrument by the base unit

3.5 Assembly

The following parts must be added to the Ettan DALTsix and Ettan DALTtwelve instruments before they can be used:

- IPG strips
- Gel Cassettes containing gels
- Buffer liquids

3.6 Connections

Communication

Connect Ettan DALTtwelve according to the electrical drawings in *Appendix A*. Connect Ettan DALTsix according to the user documentation supplied with the Power Supply.

Electrical power

Connect the power cord to a grounded power outlet specified in *Section 3.1*.

3.7 Spare parts and accessories

For correct up to date information on spare parts and accessories visit:
www.gelifesciences.com\2DE

4 Operation

**WARNING**

Check electrical cables for damage and the electrophoresis unit for damage or leakage before use. If damage and/or leakage is present, do not use the equipment. There is a risk for high voltage injuries.

**WARNING**

The high voltage power supply must always be disconnected when the safety lid of the electrophoresis unit is taken off. The high voltage power supply must never be switched on unless the safety lid is on the electrophoresis unit.

**WARNING**

Do not overfill the tank. Spillage can cause short-circuiting of internal circuits and the breakdown of electrical isolation which increases the risk of electrical shock. Always make sure the equipment is disconnected from power supply when filling the tank.

**WARNING**

Any liquid on the equipment must be dried off before connecting the power supply.

**WARNING**

Always wear gloves, protective clothing, and eye protection when handling DIGE Gels, DIGE Buffer, IPG strips, gel cassettes or any other equipment these items will come into contact with.

4.1 Operation overview

The typical workflow in Ettan DALT system, when using precast gels, can be divided into the following steps:

Step	Action	Page
1	Preparing Ettan DALT system for electrophoresis using precast gels	26
3	Equilibrating Immobiline™ DryStrip Gels	28
4	Applying equilibrated Immobiline DryStrip Gels	29
5	Inserting gels into Ettan DALT electrophoresis units	30
6	Programming the power supply/control unit	31
7	Starting the electrophoresis	37
8	Unloading the gels from the Ettan DALT electrophoresis units	37

Note: *Precast DIGE Gels have been used as an example throughout this chapter.*

4.2 Preparations before start

Preparing Ettan DALT system for electrophoresis using precast gels

Preparing Ettan DALTsix

- 1 Prepare anode assembly**
Insert the anode assembly/cassette carrier into the tank. The anode assembly is molded so that it can only be inserted in one orientation. The side edge of the assembly should fit into the slot in the side of the tank.
- 2 Fill with anode buffer**
Add one bottle (125 ml) of DIGE Anode Buffer stock solution included in the DIGE Buffer Kit into the tank. Fill the electrophoresis unit to the 4.5 l fill line with distilled or deionized water. Connect the pump to mains power supply.
- 3 Turn on the temperature controller**
Turn on the MultiTemp III temperature controller (or similar) and adjust the temperature to 22°C for a day run and to 15°C for an overnight run. Equilibrate the buffer to 15°C before starting an overnight run. Refer to the *MultiTemp III User Manual* for more information.

4 Set aside upper chamber

The upper chamber is prepared once the gel has been inserted into Ettan DALTsix. See *Inserting gels into Ettan DALT electrophoresis units*, on page 30.

For more detailed instructions, refer to *Ettan DALTsix Electrophoresis System User Manual*.

Preparing Ettan DALTtwelve

1 Prepare cathode buffer

In a separate container dilute the concentrated cathode buffer included in the DIGE Buffer Kit to working strength by adding four bottles of DIGE cathode buffer (total volume 500 ml) and fill up with distilled or deionized water to a total volume of 2.25 l.

2 Prepare anode buffer

Ensure that the valve on the separation unit is set to “circulate”. Add the entire contents of two bottles of DIGE anode buffer stock solution included in the DIGE Buffer Kit into the tank. Rinse the bottles with distilled or deionized water and pour it into the tank. Fill the tank to the 7.5 l fill line with distilled or deionized water, in this way washing the DIGE anode buffer from the buffer seal.

Note: Avoid pouring the DIGE buffer on the tubing by spreading the tubing slightly with one hand while pouring the solution with the other (Figure 4-1).



Figure 4-1. Spreading the tubing elements apart with one hand while pouring the solution with the other (to avoid pouring the DIGE buffer onto the tubing).

3 Turn on the electrophoresis unit

Turn on the electrophoresis unit by pressing the Power switch to the I position.

4 Start the pump

Start the pump to mix the buffers and set the separation unit to desired temperature. A temperature of 22°C is recommended for day runs, and 15°C for overnight runs.

Note: A temperature of 25°C is recommended for electrophoresis.

For more detailed instructions, refer to *Ettan DALTtwelve System User Manual*.

Equilibrating Immobiline DryStrip Gels

Equilibration is carried out in a two-step process to reduce vertical streaking in the second dimension. The first step saturates the IPG strip with the SDS system and the second step blocks the protein thiol groups. The equilibration solution contains buffer, urea, glycerol, reductant, SDS and dye. Tubes and volume of equilibration solution to use are specified in *Table 4-1*.

Prepare equilibration solution.

- 1 Prepare SDS equilibration buffer (see *Appendix B.1*). This is a stock solution.
- 2 Just prior to use, add 50 mg DTT per 10 ml SDS equilibration buffer (0.5% [w/v]).

Equilibration

- 1 Place the IPG strips in individual tubes with the support film toward the tube wall.
- 2 Add 10 ml DTT-containing solution to each tube.
- 3 Place the tubes on a rocker and equilibrate for 15 min.

Note: When using CyDye™ DIGE saturation dyes (*Labeling Kit for scarce samples*), repeat the first equilibration with DTT-containing SDS equilibration solution for another 15 min.

Second equilibration

A second equilibration is performed with an iodoacetamide solution (instead of DTT).

- 1 Prepare a solution of 450 mg iodoacetamide per 10 ml of SDS equilibration buffer (4.5% [w/v]).
- 2 Decant the first equilibration solution and add the same volume of iodoacetamide containing equilibration solution to each tube.
- 3 Place the tubes on a rocker and equilibrate for an additional 15 min.

Note: The subsequent steps of electrophoresis unit preparation, insertion of gels and melting of the Sealing Solution can be performed while the IPG strips are equilibrating.

Note: Be consistent with the timing of the equilibration steps.

Table 4-1. Suggested containers and volumes of equilibration solution.

Strip length (cm)	Container	Equilibration solution (ml)
7	Disposable, 15-ml conical tubes	2.5–5
11	25 × 200 mm screw-cap culture tubes	5–10
13	25 × 200 mm screw-cap culture tubes	5–10
18	25 × 200 mm screw-cap culture tubes, Equilibration tubes available from GE Healthcare, or Petri dish	10–15
24	Equilibration tubes available from GE Healthcare or Petri dish	10–15

Note: The subsequent steps of gel assembly, preparation of electrophoresis unit, insertion of the gel into the Precast Gel Cassette, and melting of the sealing solution can be performed as the Immobiline DryStrip Gels are equilibrating, as long as the time frames above are adhered to.

Applying equilibrated Immobiline DryStrip Gels



CAUTION

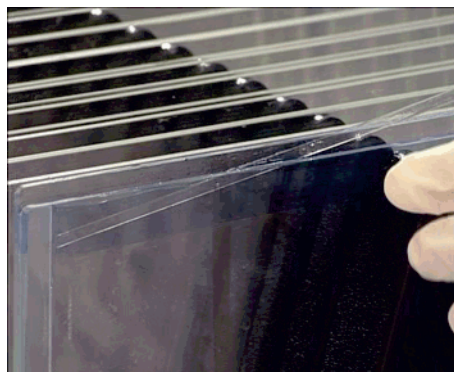
Handle the gels with care. The edges of the glass plates of the gels are sharp.

- 1 Place the gels in the Ettan DALT cassette rack.
- 2 Briefly rinse the Immobiline DryStrips by submerging them in a measuring cylinder containing SDS electrophoresis buffer (see Appendix B.3) for Ettan DALT.
- 3 Holding one end of the Immobiline DryStrip with forceps, carefully place the Immobiline DryStrip in-between the two glass plates of the gel. Using a thin plastic spacer, push against the plastic backing of the Immobiline DryStrip (not the gel itself) and slide the strip between the two glass plates until it comes into contact with the surface of the gel.

Note: The strip should just rest on the surface of the gel. Avoid trapping air bubbles between strip and the gel and avoid piercing the second-dimension gel with the strip.

Note: The acidic end of the Immobiline DryStrip should be on the right side of the gel when the shorter of the two plates is facing the user.

Note: The gel face of the strip must not touch the opposite glass plate.



- 4 Melt an aliquot of agarose sealing solution (see *Appendix B.4*) in a heating block or boiling water bath for each Immobiline DryStrips. Allow the agarose to cool slightly and slowly pipette the molten agarose solution, along the upper surface of the gel, up to the top of the glass plate. Take care not to introduce bubbles. Do not allow the agarose to solidify.
- 5 Once the agarose solution has completely set the gel should be run in the second dimension as soon as practically possible.

Inserting gels into Ettan DALT electrophoresis units



WARNING

Disconnect the unit from the power supply when inserting gels.

Inserting gels into Ettan DALTsix

When the electrophoresis buffer has reached the desired temperature, insert the loaded Gel Cassettes with the Immobiline DryStrip Gels in place.

- 1 Wet the UBC sealings with cathode buffer or 0.1% SDS (immerse the sealings in solution or spray the sealings of the UBC using a plant sprayer) and carefully slide the UBC over the gel cassettes.

Note: Do not move the UBC repeatedly up and down as this will reduce the sealing effect.

- 2 In a separate container, add 2 × 125 ml (2 bottles) of cathode buffer. Rinse the bottles and fill up with distilled or deionized water to 1.2 l.

- 3 Fill the UBC with 1.2 liters of diluted cathode buffer and use a funnel to adjust the buffer level in the lower buffer chamber to the same height as in the UBC, by adding water or diluted anode buffer.
- 4 Attach and close the lid. Connect the power leads to the power supply.

Inserting gels into Ettan DALTwelve

When the electrophoresis buffer has reached the desired temperature, insert the loaded Gel Cassettes with the Immobiline DryStrip Gels in place.

Note: *Gel Cassettes and Blank Cassette Inserts slide much more easily into the unit if they are wet. Wetting the cassette with some cathode buffer using a soaked kleenex or alternatively distilled or deionized water from a squirt bottle can be used to wet the cassettes and Blank Cassette Inserts as they are being loaded into the unit.*

- 1 Fit Blank Cassette Inserts into any unoccupied slots.
- 2 Load the unit from back to front.
- 3 When all 12 slots are occupied, the buffer level should be slightly below the level of the gaskets. If the buffer level is too low, add distilled or deionized water to the lower buffer chamber. If excess anode buffer is in the upper reservoir, remove it with a pipette. Pour the diluted cathode buffer into the tank to the fill line (some of this buffer may drip through the gasket and mix with the anode buffer during the run, but this will not affect performance or results).
- 4 Close the lid.

Programming the power supply/control unit

Programming Ettan DALTsix

Turn on the power supply/control unit according to the user documentation provided with the equipment.

Refer also to the user documentation for programming instructions.

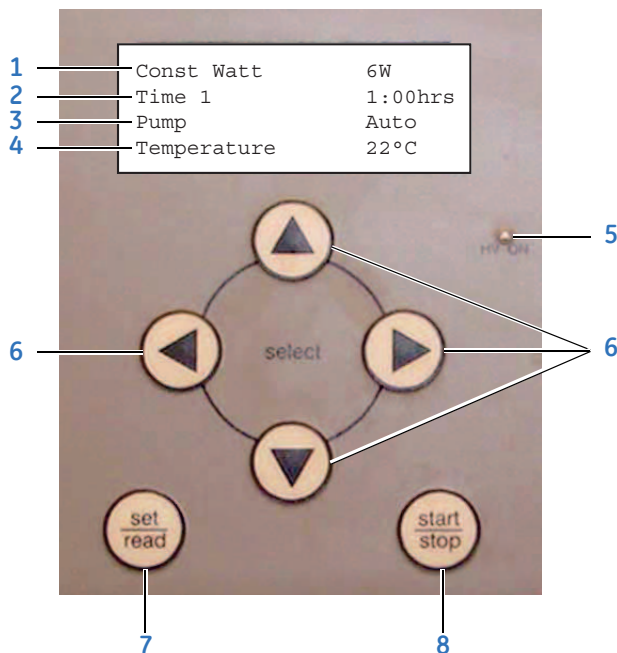
For recommended electrophoresis conditions, see *Electrophoresis conditions with precast gels*, on page 34.

Programming Ettan DALTwelve

If not already turned on, turn on the power supply/control unit by pressing the Power switch to the I position.

When the unit is turned on, the default settings of constant power, continuous run, auto pump, and 25 °C are shown on the display, see *Figure 4-2*.

The control unit has four programmable parameters: **Run Type**, **Timer**, **Pump**, and **Temperature**, see *Figure 4-2*.



Item	Description	Item	Description
1	Run type	5	HV ON indicator
2	Timer	6	Arrow buttons
3	Pump	7	set/read button
4	Temperature	8	start/stop button

Figure 4-2. Controller interface in programming mode.

The **set/read** button toggles the controller between the set and read modes. The **start/stop** button starts and stops the execution of the programmed electrophoresis run. The ◀ and ▶ buttons move the cursor between run parameters, and the ▲ and ▼ buttons change the settings of the parameters.

Below is a description of the programmable parameters (see also *Table 4-2*):

- **Run Type** determines the method of power regulation. The options for run type are constant power, constant current, constant voltage, and crossover mode. In crossover mode the voltage and current limits for the run are set manually, instead of using the instrument defaults of 600 V and 1 000 mA. As the run progresses, the power supply operates in the mode that is limiting.

- **Timer** controls the duration of the electrophoresis run. The options are continuous run, timed step, timed and hold, and volt-hours. Up to three timed steps of up to 100 h each can be programmed using the timed step mode. With timed step mode, all the steps must have the same run type.
- **Pump** controls buffer circulation through the separation unit. The options for the pump are On, Off, and Auto. Auto mode activates the pump only when power is applied.
- **Temperature** controls the cooling or heating of the buffer in the separation unit. The temperature range is from 10°C to 50°C. On the display, cooling is indicated by ↓ and heating is indicated by ↑. The pump must be on to properly cool or heat buffer in the tank. The lower temperature limit is a function of the ambient temperature and the power reading. To reach the lower temperature limit (10°C) or in laboratory environments where the ambient temperature is elevated, it may be necessary to place the unit in a cold box or cold room.

Table 4-2. Parameter options.

Parameter	Option	Display	Range
Run Type	Constant power	Const Watt	1 to 200 W
	Constant current	Const Amp	1 to 1000 mA
	Constant voltage	Const Volt	1 to 600 V
	Crossover mode	V & mA	1 to 600 V, 1 to 1000 mA
Timer	Timed step	Time 1 - Time 3	00:01 to 99:59 hrs
	Time and hold	T/Hold	00:01 to 99:59 hrs
	Volt-hours	Stop at	1 to 9999 Vhr
	Continuous run	Continuous run	-
Pump	On	On	-
	Off	Off	-
	Auto	Auto	-
Temperature	-	Temperature	10 to 50°C

See *Table 4-3* for a sample program. Twelve gels are electrophoresed at 180 W, constant power, and 25 °C with a 5 W/gel entry phase. The second phase is extended longer than required to ensure that the dye front runs off the gel.

Step	Parameter	Value
1	Const Watt	60 W
	Time 1	00:45 hrs
	Pump	Auto
	Temperature	25°C
2	Const Watt	180 W
	Time 2	04:00 hrs
	Pump	Auto
	Temperature	25°C

Table 4-3. Sample program

For recommended electrophoresis conditions, see *Electrophoresis conditions with precast gels*, on page 34.

Electrophoresis conditions with precast gels

Recommended running conditions for DIGE gels – Ettan DALTsix

The maximum rated electrical input for the electrophoresis unit is 400 mA, 100 W, and 600 V. For overnight runs we recommend to set the temperature to 15°C. Alternatively, set the temperature to 22°C.

For further run conditions, see the *DIGE Gels and DIGE Buffer Kit Short Instructions* in the DIGE Buffer Kit.

Run conditions (Day run):

Set the MultiTemp temperature to 22°C.

Step	mA/gel	Voltage (V)	W/gel	Time (hours:mins)
1	10	80	1	1:0
2	50	500	17 ¹	4:00 - 5:00 ²

¹ The maximum rated input power for the electrophoresis unit is 100 W.

² Continue the electrophoresis until the bromophenol blue reaches the end of the gel.

Run Conditions (Overnight):

Set the MultiTemp temperature to 15°C.

Step	mA/gel	Voltage (V)	W/gel	Time (hours:mins)
1	12	150	1.5	15:00 - 17:00 ¹

¹ Continue the electrophoresis until the bromophenol blue reaches the end of the gel.

Recommended running conditions for DIGE Gels – Ettan DALTtwelve

Program the Ettan DALTtwelve electrophoresis unit to deliver the following protocol.

Constant power

Temperature: 22°C

Step	Power W/gel	Duration
1	1	1 hour
2	17 (max 180) ¹	Until the bromophenol dye front reaches the bottom of the gel (approximately 4-5 hours for a full set of 12 gels).

¹ For overnight runs, the power is set to 1 W/gel and the recommended temperature is 15°C. Alternatively, set the temperature to 22°C. Run for 1 hour and then increase to 1.5 W/gel.

Recommended running conditions for DALT Precast Gels and 1 mm Laemmli gels – Ettan DALTsix

Run conditions (Day run):

Applicable to 1 mm thick 12% PAA gel and Laemmli Buffer system. Set the MultiTemp temperature to 25°C.

Step	mA/gel	Voltage (V)	W/gel	Time (hours:mins)
1	10 ¹	80	1	1:00
2	40 ¹	500	13	4:30 - 6:00 ²

¹ For 1.5 mm gels, increase the current by 50%.

² Continue the electrophoresis until the bromophenol blue reaches the end of the gel.

Run Conditions (Overnight):

Set the MultiTemp temperature to 30°C.

Step	mA/gel	Voltage (V)	W/gel	Time (hours)
1	10 ¹	80	1	1
2	12 ¹	150	2	15 - 17 ²

¹ For 1.5 mm gels, increase the current by 50%.

² Continue the electrophoresis until the bromophenol blue reaches the end of the gel.

Recommended running conditions for DALT Precast Gels and 1 mm Laemmli gels – Ettan DALTtwelve

Electrophoresis is performed at constant power in two steps. Stop electrophoresis when the dye front is approximately 1 mm from the bottom of the gel.

Temperature control improves gel-to-gel reproducibility, especially if the ambient temperature of the laboratory fluctuates significantly.

For best results, gels should be run at 25°C. For overnight runs we recommend to set the temperature to 30°C.

	Step	Power (W/gel)	Approximate run duration (h:min)
Day runs:			
(set temperature to 25 °C)			
1-mm-thick gels (lab-cast and precast)	1	2	0:45
	2	17 (max 180)	4:00
1.5-mm-thick gels	1	5	0:30
	2	17 (max 180)	6:00
Overnight runs:			
(set temperature to 30 °C, power supply for continuous run)			
1.0-mm-thick gels run overnight ¹		1	18:00
1.5-mm-thick gels run overnight ¹		1.5	17:00

¹ For the best possible resolution, faster separation times should be used. Use the faster (< 6 h) protocols instead.

4.3 Performing a run

For Ettan DALTsix

For instructions on how to start and stop the electrophoresis program, refer to the user documentation for the power supply/control unit used.

For Ettan DALTwelve

- 1 Press the **start/stop** button on the power supply/control unit to start the electrophoresis program.
- 2 To stop the electrophoresis program before it is finished, press the **start/stop** button.

4.4 Procedures after a run

Unloading the gels from the Ettan DALT electrophoresis units



WARNING

Disconnect all units from mains power supply before removing the safety lid, unloading the gels, draining and cleaning the electrophoresis unit.

Unloading the gels in Ettan DALTsix



CAUTION

Ettan DALTsix electrophoresis unit is heavy, especially when filled with buffer. Handle the unit with care to avoid personal injury.

- 1 Carefully pull the upper buffer chamber upward using the handles at each end.
- 2 As the upper buffer chamber is pulled upward make sure that the cassettes remain in the anode assembly.
- 3 Take out the anode assembly.
- 4 Remove the Gel Cassettes from the anode assembly.
- 5 Open the Gel Cassettes using a Wonder Wedge to separate the plates on the side opposite the hinge.

4 Operation

4.4 Procedures after a run

- 6 For lab-cast gels, run an edge of the Wonder Wedge down each side of the cassette along the spacer and carefully lift the gel out of the cassette. For precast gels, lift the gel out by grasping the GelBond backing.
- 7 Tilt the entire electrophoresis unit on the side of the unit (do NOT tilt to front or back) to empty the buffer from the lower buffer chamber into a sink or a waste container. See Section 3.3 for instructions of how to place the unit for safe use.
- 8 Rinse all the components with distilled or deionized water. Flush the pump with distilled or deionized water by filling the unit and turning the pump on.

Unloading the gels in Ettan DALTwelve

- 1 After the run has been completed, remove one or more of the Blank Cassette Inserts or gels and drain enough of the electrophoresis buffer from the tank to expose 2–3 cm of the Gel Cassettes. This will ease removing the remaining cassettes. When the first cassette, either blank or gel, is removed, a sucking sound will be heard as air is drawn into the lower chamber. For runs of six or fewer gels, alternating Gel Cassettes with blank cassettes also eases the removal of the Gel Cassettes at the end of a run. Leave enough of the electrophoresis buffer in the tank to act as a lubricant between the glass cassettes and the buffer seal.

There are two methods for removing the first cassettes from the unit: using the cassette removal tool or the hands:

- **Using the cassette removal tool:** Carefully insert the cassette removal tool between the cassette and the buffer seal, with the folded tip facing the cassette, until the tip is beneath the bottom edge of the cassette. Verify that the tool is caught on the bottom edge of the cassette, then lift it out slowly with the tool (*Figure 4-3*).



Figure 4-3. Using the cassette removal tools.

- **Using the hands:** By hand, apply upward pressure alternately to each side of the cassette, gradually shifting it up until you can grasp it and remove it (*Figure 4-4*).



Figure 4-4. Removing cassettes by hand.

- 2 Open the cassettes using a Wonder Wedge and carefully transfer the gels to a staining tray, for example. Take care to ensure that the gel does not adhere to the spacers.

Note: *Vinyl gloves are less sticky than latex gloves and make it easier to handle large gels.*

Detection

Scan the gels as soon as possible after the second dimension SDS-PAGE is finished in order to minimize protein diffusion. Typhoon™ Variable Mode Imager or Ettan DIGE Imager are recommended for scanning DIGE second dimension SDS-PAGE gels. Store the gels in a refrigerator in a closed container and keep the gels moist. Allow the gels to reach room temperature before scanning. Keep the gels in the glass cassettes throughout scanning.

Cleaning the Ettan DALT units after a run

Cleaning Ettan DALTsix

- 1 When all of the gels and Blank Cassette Inserts have been removed, tilt the entire electrophoresis unit on the side of the unit (do not tilt to front or back) to empty the buffer from the lower buffer chamber into a sink or a waste container. See *Section 3.3* for instructions of how to place the unit for safe use.
- 2 Rinse all the components with distilled or deionized water. Flush the pump with distilled or deionized water by filling the unit and turning the pump on.

Cleaning Ettan DALTtwelve

- 1 When all of the gels and Blank Cassette Inserts have been removed, drain the buffer by turning the pump valve to drain with the pump on. Emptying will take about 1 min.
- 2 After the buffer has been removed, pour 3–4 l of distilled or deionized water into the unit and allow it to drain. Rinse the unit with 5–7 l of distilled or deionized water in circulate mode, empty again, and repeat if necessary.
- 3 Remove the lid from the unit by sliding it to the left and rinse it with distilled or deionized water. Slide the lid back on its hinges before using the unit again.

5 Maintenance



WARNING

Disconnect power. Always disconnect power from the instrument before performing any maintenance task.



WARNING

Hazardous substances. When using hazardous chemical and biological agents, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation and maintenance of the system.

5.1 User maintenance schedule

Before each run check the following:

- All parts are clean
- All parts are intact and free of cracks and other damage. If a part is damaged replace the part or entire instrument before use.
- The power cord and high voltage cable is intact and free of cracks and other damage. If the cable is damaged replace it before use.
- There is no leakage from parts containing buffer or cooling water. Moist or liquid must be removed from the equipment before use.
- The circulation pump spins and that there is circulation of liquid.

5.2 Cleaning

For day-to-day operation of the unit, the cleaning procedure outlined in unit operation—thoroughly rinsing the separation tank with distilled or deionized water—is sufficient. If desired, the internal components of the separation unit can be removed for a more thorough cleaning (see below). The unit can also be periodically cleaned with a dilute solution of a mild detergent.

Clean the gel casting cassettes and pre-cast gel cassettes with a dilute solution of a laboratory cleanser such as RBS-35, from Pierce Chemical Company. Rinse the cassettes thoroughly with distilled or deionized water.



NOTICE

Do not autoclave or heat any part above 50 °C.



NOTICE

Do not expose the unit or its parts to organic solvents, including >20% ethanol.



NOTICE

If using radioactive reagents, decontaminate the unit with a cleaning agent such as CONTRAD 70 or Decon™ 90 from Decon Laboratories, Inc.

5.3 Component maintenance



WARNING

Electrical Shock Hazard. Do not use the equipment if any part is cracked or broken.

If any of the components become cracked or broken it should be replaced. The upper buffer chamber (UBC) should be replaced if any of the rubber flaps become cut or torn.

To prevent damage to the flaps, ensure that all sharp edges on glass plates are smoothed and exercise care when using the cassette removal tool.

With normal use the UBC ribs may become bowed upward; this bowing should not affect the performance of the seal.

To remove the anode plate or any of the other internal components, follow the directions below.

- 1 Insert the buffer seal removal tools through the fifth or sixth slot, with the wider end underneath the tubes. Turn the tools 90° and place as close to the end blocks as

possible. Carefully pull upward on the buffer seal until it is removed from the unit (see Fig 5-1).



Figure 5-1. Using the buffer seal removal tools

- 2 Slide the two baffle plates upward and out of the unit.
- 3 Remove the two flow guides by pulling out the retaining pins and lift the blocks out.
- 4 Remove the anode plate by unscrewing the flathead nylon screw and lifting the plate out.
- 5 To reassemble the unit, replace the anode plate and screw, making sure that the sealing sleeve is in place. Spread a small amount of GelSeal compound on the plug with a swab applicator to prevent corrosion.
- 6 Replace the flow guides and baffle plates.
- 7 Put a light film of GelSeal compound on the gasket of the buffer seal and reinsert it into the unit using even pressure. Make sure that it is fully seated before using the unit.

5 Maintenance

5.3 Component maintenance

6 Troubleshooting

6.1 Electrical and mechanical

Symptom	Possible cause	Corrective action
No current at start of run.	Insufficient volume of buffer in upper reservoir.	Ensure that the unit contains enough buffer to contact the upper electrode.
Buffer not circulating.	Pump is not primed.	Turn circulation valve to Drain to fill with buffer then back to circulate.
	Pump is off or set to Auto .	Set unit to ON on control unit.
	Pump is broken.	Contact GE Healthcare Service.
Display on the control unit blank (Ettan DALTwelve only).	Unit is not turned on.	Turn unit on with power switch.
	Unit is not plugged in.	Plug in unit.
	Display is broken.	Contact GE Healthcare Service.
	Control unit display malfunction.	Turn the AC power switch off for a few seconds, then on again. If the problem persists, call GE Healthcare Service.
Open-circuit warning (Ettan DALTwelve only).	Safety interlocks not engaged.	Make sure that the lid is completely closed. Make sure that the pump valve is turned completely to circulate. If this does not correct the error, contact GE Healthcare Service.

6.2 Pre-cast gels

Symptom	Possible cause	Corrective action
Second-dimension separation proceeds slowly with high current.	All of the slots in the buffer seal are not occupied by either gel cassettes or blank cassettes.	Ensure that all 12 slots in the buffer seal are occupied. Do not pour more than the suggested volume (7.5 l) into the lower reservoir.
	Anodic buffer has mixed with cathodic buffer from overfilling of either the cathodic or the anodic reservoir.	Ensure that the level of the anode (lower) buffer does not come above buffer seal when the separation unit is fully loaded. Remove any excess anode buffer from the upper reservoir. Ensure that the level of cathode buffer is not above the air vents in the upper reservoir.
Dye front is irregular.	The top surface of the gel has been damaged during application of the IPG strip.	Take care during application of the IPG strip that neither gel is damaged.
	Bubbles or liquid between the gel and the glass plate.	Use the roller to remove any bubbles or excess liquid between the gel and the glass plate. Ensure that no visible bubbles remain and that the gel adheres firmly to the glass and resists movement.
	Interfering substances in the first dimension.	Contaminants in the sample can cause distortions or swollen regions in the IPG strip following IEF. Modify sample preparation to limit these contaminants. Refer to <i>2-D Electrophoresis: Principles and Methods</i> .
Pronounced downward curving of the dye front on one side of the gel.	There is an unfilled gap between the gel and one of the spacers.	When sealing the IPG strip into place, ensure that some of the agarose sealing solution flows down any gap that may exist between the gel and the spacer.

6.3 Stained gels

Symptom	Possible cause	Corrective action
Protein spots are diffuse or broader than usual.	Poor quality reagents.	Use only the highest-quality reagents.
	Incomplete polymerization.	Make sure that polymerization is complete.
	Incorrect equilibration time.	Check equilibration time of IPG strips. Too long can lead to diffusion, and too short can lead to incomplete equilibration.
	Gel surface damaged by IPG strip.	Make sure the IPG strip rests on the slab gel surface without damaging.
	Problems with first dimension.	Refer to troubleshooting guides for IPGphor™ or Multiphor™ units, or <i>2-D Electrophoresis: Principles and Methods</i> .
Protein spots are poorly resolved.	Incomplete polymerization.	Make sure that polymerization is complete.
	Diffusion of proteins.	Begin electrophoresis as soon as the IPG strips are loaded, to prevent diffusion of low molecular- weight proteins.
	Running too fast.	Reduce the power, current, or voltage.
	Too high temperature.	Reduce the temperature setting.
	Problems with first dimension.	Refer to troubleshooting guides for IPGphor™ or Multiphor™ units, or <i>2-D Electrophoresis: Principles and Methods</i> .
Smeared or comet shaped spots (Ettan DALTsix only).	Buffer problems.	Check pH of cathode buffer. Should be between 8.3 and 8.8. Make sure that 2× Laemmli buffer is used in the upper (cathode) chamber. Buffer or SDS depleted.

6 Troubleshooting

6.3 Stained gels

Symptom	Possible cause	Corrective action
Protein spots are near the buffer front.	Incorrect pore size.	Pore size of the gel is too large. Increase the %T.
	Degraded proteins.	Proteins degraded during sample preparation. Add protease inhibitors during sample preparation.
	Incorrect pH.	Check the pH of the 4× gel buffer. It should be pH 8.8. Proteins will migrate faster below pH 8.8.
Protein spots have not entered the gel when buffer front has reached the bottom of the gel.	Incorrect pore size.	The gel pore size is too small. Decrease the %T.
	Incorrect pH.	Check the pH of the 4× gel buffer. It should be pH 8.8. Proteins will migrate more slowly above pH 8.8.
Protein spots are at both extremes but not in center.		The molecular weight range of the sample requires an acrylamide concentration gradient to resolve the full range of proteins.
Vertical protein streaks.	IPG strip not properly placed on gel surface.	Make sure IPG strip uniformly contacts the gel surface along its entire length. Avoid gouging the surface of the separating gel.
Spots skewed or distorted.	Gels run too fast – uneven migration.	Run at a lower power setting. Use a two-step program: Start at a low power setting until the proteins enter the gel, then increase the power for the remainder of the run.
	Uneven gel surface.	Overlay the running gel with water-saturated butanol before polymerization begins to avoid forming an uneven gel surface. Uneven gel polymerization or gradient formation.
Heavy background after silver staining.		Use reagents of the highest purity, preferably electrophoresis grade. Use deionized, double-distilled water.

Symptom	Possible cause	Corrective action
Distortion in the 2-D pattern.	Bubbles or liquid between the gel and the glass plate.	Use the roller to remove any bubbles or excess liquid between the gel and the glass plate. Ensure that no visible bubbles remain and that the gel adheres firmly to the glass and resists movement.
	Interfering substances in the first dimension.	Contaminants in the sample can cause distortions or swollen regions in the IPG strip following IEF. These distortions can result in disturbances in the second dimension.
Vertical gap in the 2-D pattern.	Bubble between IPG strip and top surface of second dimension gel.	Ensure that no bubbles are trapped between the IPG strip and the top surface of the second-dimension gel.
Vertical streaking.	Incorrectly prepared equilibration solution.	Prepare equilibration solution according to instructions.
	Poor transfer of protein from IPG strip to second dimension gel.	Use low power for sample entry phase. Extend entry phase if necessary.
	Insufficient equilibration.	Prolong equilibration time.
Spots are vertically doubled, or "twinned".	IPG strip is not placed properly.	Ensure that the plastic backing of the IPG strip is against the glass plate of the second-dimension cassette.
Poor representation of higher molecular weight proteins.	Incorrectly prepared equilibration solution.	Prepare equilibration solution according to instructions.
	Poor transfer of protein from IPG strip to second dimension gel.	Use low power for sample entry phase. Extend entry phase if necessary.

6 Troubleshooting

6.3 Stained gels

7 Reference information

7.1 Specifications

Parameter	Value
Ingression protection	IP20
Supply voltage	100 to 240 V ~, 50 to 60 Hz
Power consumption: Ettan DALTsix Ettan DALTwelve	100 W 650 W
Fuse specifications	T2.5 A 250V
Dimensions (H × W × D)	160 × 278 × 471 mm
Weight: Ettan DALTsix Ettan DALTwelve	8.3 kg 11.1 kg 24.5 kg
Ambient temperature	+4 to +40 °C
Relative humidity tolerance	0 to 90%
Atmospheric pressure	84 to 106 kPa (840 to 1060 mbar)
Acoustic noise level	< 70 dB A

7.2 Literature

For further information regarding the Ettan DALTsix and Ettan DALTtwelve Systems, refer to the following:

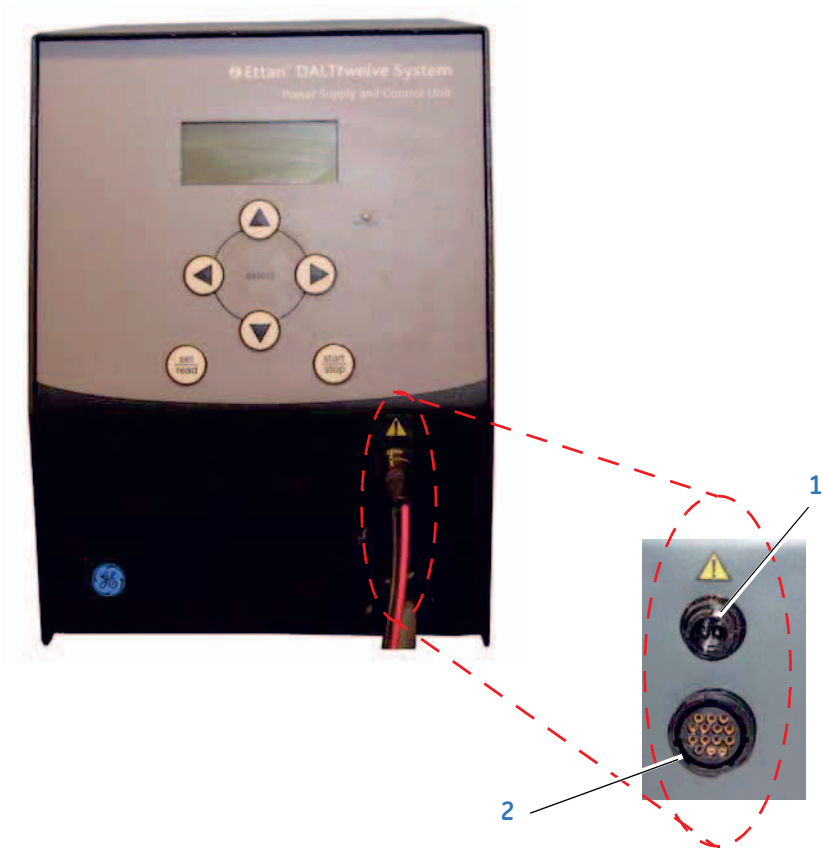
- *Ettan DALTsix Electrophoresis System User Manual*
- *Ettan DALTtwelve System User Manual*
- *Ettan DIGE System User Manual*
- *2-D Electrophoresis: Principles and Methods*
- *DIGE Gels and DIGE Buffer Kit Short Instructions*
- *MultiTemp III User Manual*

7.3 Ordering information

For ordering information visit www.gelifesciences.com/2DE.

Appendix A Electrical and communication connections

A.1 Front of Ettan DALTwelve Power Supply/Control unit



Part	Description	Part	Description
1	High voltage connector	2	Signal and power cable connector

A.2 Rear of Ettan DALTwelve Power Supply/Control unit



Part	Description	Part	Description
1	Power switch	2	Power inlet

Appendix B Recipes

B.1 SDS equilibration buffer solution

(6 M urea, 50 mM Tris-HCl pH 8.8, 30% glycerol, 2% SDS, 0.001% bromophenol blue, 200 ml)¹

	Final concentration	Amount
Urea (FW 60.06)	6 M	72.07 g
Tris-HCl, pH 8.8 (see <i>Appendix B.2</i>)	50 mM	6.7 ml
Glycerol (87% w/w)	30% (v/v)	69 ml (84.2 g)
SDS (FW 288.38)	2% (w/v)	4.0 g
1% Bromophenol blue stock solution	0.001% (w/v)	2 mg
Double-distilled water	—	to 200 ml

¹ This is a stock solution. Just prior to use, add DTT or iodoacetamide (for first or second equilibration, respectively) as described in the in *Equilibrating Immobiline DryStrip Gels*, on page 28.

Store in 40 ml aliquots at -20 °C.

B.2 4× resolving gel buffer solution

(1.5 M Tris base, pH 8.8, 1 l)

	Final concentration	Amount
Tris base (FW 121.1)	1.5 M	181.7 g
Double-distilled water	—	750 ml
HCl _{aq}	—	adjust to pH 8.8
Double-distilled water	—	to 1 l

B.3 SDS electrophoresis buffer

(25 mM Tris base, 192 mM glycine, 0.2% SDS, 20 l)¹

	Final concentration	Amount
Tris base (MW 121.14)	25 mM	60.5 g
Glycine (MW 75.07)	192 mM	288.0 g
SDS (MW 288.38)	0.2% (w/v)	40.0 g
Double-distilled water	—	to 10 l

¹ The pH of this solution should not be adjusted.

Store at room temperature.

B.4 Agarose sealing solution

(25 mM Tris base, 192 mM glycine, 0.1% SDS, 0.5% agarose, 0.002% bromophenol blue, 100 ml)

	Final concentration	Amount
Laemmli SDS electrophoresis buffer (see <i>Appendix B.3</i>)		100 ml
Agarose (NA or M)	0.5%	0.5 g
1% Bromophenol blue stock solution	0.002% (w/v)	200 µl

Add all ingredients into a 500-ml Erlenmeyer flask. Swirl to disperse. Heat in a microwave oven on low or on a heating stirrer until the agarose is completely dissolved. Do not allow the solution to boil over. Dispense 1.5-ml aliquots into screw-cap tubes and store at room temperature.

For local office contact information, visit
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First published Nov. 2009.

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