Density Gradient Fractionation Systems

Installation and Operation Guide

TELEDYNE ISCO
A Teledyne Technologies Company

#69-3873-246

Revision A, February 18, 2010.

Foreword

This instruction manual is designed to help you gain a thorough understanding of the operation of the equipment. Teledyne Isco recommends that you read this manual completely before placing the equipment in service.

Although Teledyne Isco designs reliability into all equipment, there is always the possibility of a malfunction. This manual may help in diagnosing and repairing the malfunction.

If the problem persists, call or e-mail the Teledyne Isco Technical Service Department for assistance. Simple difficulties can often be diagnosed over the phone.

If it is necessary to return the equipment to the factory for service, please follow the shipping instructions provided by the Customer Service Department, including the use of the **Return Authorization Number** specified. **Be sure to include a note describing the malfunction.** This will aid in the prompt repair and return of the equipment.

Teledyne Isco welcomes suggestions that would improve the information presented in this manual or enhance the operation of the equipment itself.

Teledyne Isco is continually improving its products and reserves the right to change product specifications, replacement parts, schematics, and instructions without notice.

Contact Information

Customer Service

Phone:	(800) 228-4373	(USA, Canada, Mexico)
	(402) 464-0231	(Outside North America)
Fax:	(402) 465-3022	
Email:	IscoCSR@teledyne.com	

Technical Support

Phone:	(800) 775-2965	(Analytical)
	(866) 298-6174	(Samplers and Flow Meters)
Email:	IscoService@teledyne.com	
Return equipment to:	4700 Superior Street, Lincoln, NE 68504-1398	

Other Correspondence

Mail to:	P.O. Box 82531, Lincoln, NE 68501-2531
Email:	IscoInfo@teledyne.com
Web site:	www.isco.com

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General Warnings

Before installing, operating, or maintaining this equipment, it is imperative that all hazards and preventive measures are fully understood. While specific hazards may vary according to location and application, take heed of the following general warnings:

⚠ WARNING

Liquids associated with this instrument may be classified as carcinogenic, biohazard, flammable, or radioactive. Should these liquids be used, it is highly recommended that this application be accomplished in an isolated environment designed for these types of materials in accordance with federal, state, and local regulatory laws, and in compliance with your company's chemical/hygiene plan in the event of a spill.

⚠ AVERTISSEMENT

Eviter de répandre des liquides dangereux. Les liquides qui sont analysés dans cet instrument peuvent être cancérigènes, hasards biologiques, inflammables, ou radioactifs. Si vous devez utiliser tels liquides, il est très recommandé que vous le faites à l'intérieur d'un environnement isolé conçu pour tels liquides. Cet environnement isolé devrait être construit selon les règlements fédéraux, provinciaux, et locaux, aussi que le plan de votre compagnie qui concerne l'évènement d'un accident avec les matières hasardeuses.

⚠ WARNING

Avoid hazardous practices! If you use this instrument in any way not specified in this manual, the protection provided by the instrument may be impaired.

⚠ AVERTISSEMENT

Éviter les usages périlleux! Si vous utilisez cet instrument d'une manière autre que celles qui sont specifiées dans ce manuel, la protection fournie de l'instrument peut être affaiblie; cela augmentera votre risque de blessure.

⚠ WARNING

If this system uses flammable organic solvents, Teledyne Isco recommends that you place this system in a well-ventilated environment, designed for these types of materials. This environment should be constructed in accordance with federal, state, and local regulations. It should also comply with your organization's plan concerning chemical and hygiene mishaps. In all cases use good laboratory practices and standard safety procedures.

⚠ AVERTISSEMENT

Ce système peut utiliser des dissolvants organiques inflammables. Pour réduire le péril qui peut être causé par l'accumulation des vapeurs explosives, Teledyne Isco recommande que vous installez ce système dans un environnement bien-aéré qui est conçu pour les matières hasardeuses. Cet environnement devrait être construit selon les règlements fédéraux, provinciaux, et locaux. Aussi, il devrait se conformer au plan de votre organisation qui concerne les mésaventures de l'hygiène ou de chimique. En tout cas, utilisez toujours de pratiques bonnes de la laboratoire et des procédures standardes de la sûreté.

Hazard Severity Levels

This manual applies *Hazard Severity Levels* to the safety alerts, These three levels are described in the sample alerts below.

⚠ CAUTION

Cautions identify a potential hazard, which if not avoided, may result in minor or moderate injury. This category can also warn you of unsafe practices, or conditions that may cause property damage.

⚠ WARNING

Warnings identify a potentially hazardous condition, which if not avoided, could result in death or serious injury.

⚠ DANGER

DANGER – limited to the most extreme situations to identify an imminent hazard, which if not avoided, will result in death or serious injury.

Hazard Symbols

The equipment and this manual use symbols used to warn of hazards. The symbols are explained below.

Hazard Symbols	
Warnings and Cautions	
	The exclamation point within the triangle is a warning sign alerting you of important instructions in the instrument's technical reference manual.
	The lightning flash and arrowhead within the triangle is a warning sign alerting you of "dangerous voltage" inside the product.
Symboles de sécurité	
	Ce symbole signale l'existence d'instructions importantes relatives au produit dans ce manuel.
	Ce symbole signale la présence d'un danger d'électocution.
Warnungen und Vorsichtshinweise	
	Das Ausrufezeichen in Dreieck ist ein Warnzeichen, das Sie darauf aufmerksam macht, daß wichtige Anleitungen zu diesem Handbuch gehören.
	Der gepfeilte Blitz im Dreieck ist ein Warnzeichen, das Sei vor "gefährlichen Spannungen" im Inneren des Produkts warnt.
Advertencias y Precauciones	
	Esta señal le advierte sobre la importancia de las instrucciones del manual que acompañan a este producto.
	Esta señal alerta sobre la presencia de alto voltaje en el interior del producto.

Density Gradient Fractionation Systems

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Density Gradient Fractionation Systems

Section 1 Introduction

After spinning the centrifuge tubes, the Density Gradient Fractionation systems allow you to fractionate and quantitate centrifuged zones with unmatched precision. The Density Gradient Fractionation systems produce a continuous UV absorbance profile as the gradient is collected in precisely measured fractions. Fractionation is performed by introducing a dense chase liquid into the bottom of the centrifuged tube, raising the gradient intact by bulk flow. Chase solution is injected by piercing the bottom of the tube.

⚠ CAUTION

Tubes made of materials such as polycarbonate and glass cannot be pierced.

⚠ WARNING

Avoid hazardous practices! If you use this instrument in any way not specified in this manual, the protection provided by the instrument may be impaired; this will increase your risk of injury.

1.1 System Features

Density Gradient Fractionation systems are available with Teledyne Isco's Foxy R1 or Retriever 500 fraction collectors. Refer to Figure 1-1 and Table 1-1 for an overview of the features of both systems.

The system with the Retriever 500 fraction collector collects all fluids from the fractionation run, and can cut peaks using the slope-based peak separation feature of the UA-6 detector.

The system with the Foxy R1 fraction collector provides advanced peak detection and collection options. The fraction collector can analyze the analog peak signal from the UA-6 detector and cut fractions using threshold, slope, time windows, or a combination of these options. A programmable diverter valve can collect all fluids or divert non-peak fluids to a waste container.

Figure 1-1 Density Gradient Fractionation System (shown with Foxy R1 fraction collector)

Table 1-1 System Features

Item No. (Fig 1-1)	Catalog Number	Name and Description
1	68-1610-010	The **Tris Peristaltic Pump** pumps the chase liquid.
2	60-3877-060	**Tube Piercer Stand** accommodates most common centrifuge tubes. The stand allows you to upwardly displace the gradient and material. This is done by directing a chase solution through a hole pierced in the tube bottom.
3	60-0084-054	A **Density Gradient Flow Cell** is mounted at the top of the tube piercer. The system ships with a 5 mm flow cell. A 2mm flow cell option is available.
4	68-1140-006	The **Optical Unit** is also mounted above the tube piercer. The optical unit can be configured for either 254 or 280 nm wavelengths using the supplied filters.
5		The **Fraction Collector** (Foxy R1 shown) provides hands-off fraction collection. Peaks are cut as new fractions to isolate UV-absorbing material.
	68-3870-011 or	The **Foxy R1** fraction collector includes a 1.5 mL microcentrifuge tube collection rack.
	68-3880-001	The **Retriever 500** fraction collector includes tube racks for 10 to 16 mm tubes.
6	68-0940-016	**UA-6 Detector** to detect UV-absorbing material passing through the flow cell. The UA-6 includes a chart recorder.
7	69-2183-001	The **Organizer Shelf** conserves bench space.
Items Not shown		
Interface Cables	69-2134-172, or 60-1020-204	Foxy R1 to Tris pump Retriever 500 to Tris pump
	69-2134-173, or 60-1020-217	Foxy R1 to UA-6 detector Retriever 500 to UA-6 detector
Tubing and Hardware Kits	—	As supplied with the Tris pump and fraction collector.
Manuals	—	Supplied for the fraction collector, UA-6 detector, and Tris pump

☑ ***Note***

The systems do not form gradients in centrifuge tubes.

1.2 Specifications

Specifications for the system are presented in Table 1-2.

Table 1-2 System Specifications[a]

Overall Dimensions	Height: 61.7 cm (24.3) Width: 57.2 cm (22.5") Depth: 44.5 cm (17.5")
Weight	With Foxy R1: 28.7 kg (63.25 lbs) With Retriever 500: 24.6 kg (54.25 lbs) Does not include tube piercer, racks, tubes, and fluids
Power Requirements 	100 ±10 VAC, 4.9 amperes 120 ±12 VAC, 4.9 amperes 230 ±23 VAC, 2.4 amperes
Line Frequency	50 or 60 Hz
Ambient Temperature	20 to 40 °C (maximum temperature must be at least 10 °C above the boiling point of the lowest boiling solvent)
Humidity (when connected to power)	95% relative humidity maximum at 20 to 40 °C
Flow Rate Range	0.1 to 25 mL/min
Pump Speed Accuracy	±5% of full speed
System Pressure	0 to 2.7 bar
CE Conformity Specifications[b]	Pollution degree: 2 Installation category: II Maximum altitude: 2000 meters

a. Refer to the individual component manuals for additional specifications.

b. Refer to the CE Declaration of Conformity at the back of the individual component manuals for applicable standards and test results. The maximum altitude rating is per European Norm 61010-1, which establishes safety requirements for electrical equipment. The rating pertains to electrical creepage and clearances. The altitude rating is not applicable to system performance.

1.3 About this Manual

This manual covers the installation and operation of Density Gradient Fractionation systems with both available fraction collectors. While completing the installation and operation steps that relate to a fraction collector, complete only the sections for the fraction collector you received with your system. For example, if you received the Foxy R1 fraction collector with your system, skip all instructions that relate to the Retriever 500 fraction collector.

1.4 For Additional Information

Complete descriptions and operating instructions are beyond the scope of this installation guide. Refer to the instruction manuals supplied in the system manual for the individual components.

Contact your local sales representative for further assistance with the Density Gradient Fractionation system.

Density Gradient Fractionation Systems

Section 2 Installation

This section will cover setup of Isco Density Gradient Fractionation systems with the Foxy R1 and Retriever 500 fraction collectors, including the connection of electrical and interface cables and system plumbing.

2.1 Unpacking

The system is shipped in multiple cartons. Carefully unpack the shipment and inspect the contents.

⚠ CAUTION

Do not lift the Foxy R1 fraction collector by the arm.

If there is any damage to the shipping carton or any components, contact the shipping agent and Teledyne Isco (or its authorized representative) immediately.

⚠ WARNING

If there is any evidence that the system has been damaged in shipping, do not plug it into AC power. Contact Teledyne Isco or its authorized representative for advice.

Compare the contents of the boxes with the enclosed packing slips. If there are any shortages, contact Teledyne Isco immediately.

2.2 System Assembly

The Density Gradient Fractionation system is composed of several instruments. Before these instruments may be used as a system, you must first assemble the instruments and perform preliminary checkout procedures. The instructions for these steps are found in the individual instrument user manuals.

The following sections specify the minimum assembly and checkout steps that should be accomplished before proceeding with your system installation as described in the sections titled *Installation of System with Foxy R1 Fraction Collector*, on page 4, or *Installation of System with Retriever 500 Fraction Collector*, on page 8.

Unless otherwise necessary to complete the instructions in the individual manuals, electrical cables and plumbing connections should only be completed when specified in this manual.

2.2.1 UA-6 Detector and Optical Unit Preparation

The UA-6 detector and the Type 11 optical unit must be prepared as described in section 2 of the UA-6 manual.

1. Install the pen and paper.
2. The optical unit was shipped with a filter in place for UV detection at 254 nm. If you desire a different wavelength, refer to the section on Changing Wavelengths with the Type 11 Optical Unit.

The final preparation of the flow cell and optical unit is accomplished later in Section 2.2.4 of this manual.

2.2.2 Foxy R1 Fraction Collector

The Foxy R1 fraction collector should be prepared according to section 2 of the *Foxy R1 And R2 Fraction Collectors* user manual.

1. Perform the preliminary checkout procedure.
2. Install the rack and tubes.
3. Adjust the diverter valve height.

2.2.3 Retriever 500 Fraction Collector

The Retriever 500 fraction collector should be prepared according to section 2 of the *Retriever 500 Fraction Collector* user manual.

1. Perform the set up steps including automatic stop, support rod installation, and drop counter adjustment. tubes should be during these steps.
2. Perform the preliminary checkout procedure.

2.2.4 Tube Piercer Preparation

The tube piercer preparation requires a few steps. Attach the flow cell to the top plate of the Tube Piercer. Lastly, attach the optical unit to the flow cell.

Optional Aperture

Typically, the shipped flow cell has a 2 or 5 mm optical pathlength and a 1 mm height aperture. This combination is suitable for many density gradient applications and no changes should be necessary. (The flow cell's pathlength was selected when the system was ordered.)

> ☑ ***Note***
>
> If during your initial runs you find that the materials of interest absorb very little UV light, you can choose to illuminate a larger volume, refer to Section 2.5 to install a 2.8 mm aperture.

Attaching the Flow Cell

1. Attach the flow cell to the tube piercer (Figure 2-1):
   a. Remove the collar retaining nut from the flow cell.
   b. Remove the locking nut from the flow cell.
   c. Insert the flow cell into the top plate of the tube piercer.
   d. Reinstall the locking nut and hand-tighten.
   e. Reinstall the collar retaining nut and hand-tighten.

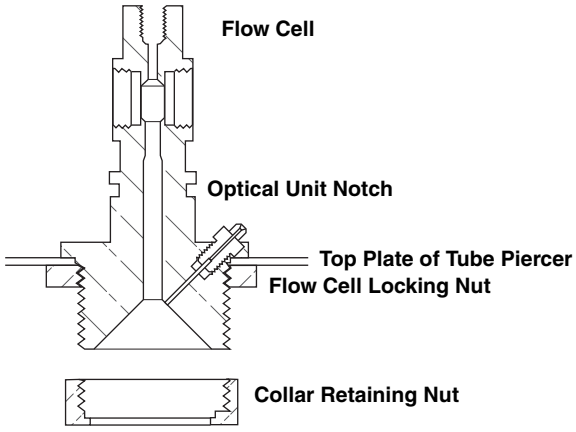


Figure 2-1 Attaching flow cell to tube piercer

2. Attach the optical unit around the flow cell.
   a. The front face of the optical unit has two white catches. Release the catch on the left side (measuring cell side). This will allow the optical unit to swing open.
   b. Note the notch near the base of the flow cell. Hold the optical unit so that the case bottom will fit in this notch and then close the optical unit.
   c. Secure the optical unit with the catch.

2.3 Installation of System with Foxy R1 Fraction Collector

1. Position system components on the organizer shelf as shown in Figure 2-2.
2. Ensure that the UA-6 detector power switch is in the OFF position.
3. Set the Tris pump CCW/STOP/CW switch to the STOP position.

Interface cable connections (Figure 2-2)

4. Locate cable 69-2134-173 that was shipped with the system components.
   a. At the back panel of the fraction collector, connect the 8-pin mini-DIN connector to the connector labeled DETECTOR.
   b. At the back panel of the UA-6 detector, connect the 9-pin connector labeled FRACTION COLLECTOR. Connect the banana plug connectors between the GROUND (center) and the 1V Recorder connections.
5. Locate cable 69-2134-172 that was shipped with the system components.
   a. At the back panel of the fraction collector, connect the 6-pin mini-DIN connector to the connector labeled PUMP.
   b. At the front panel of the Tris pump, connect the cable to the connector labeled SIGNAL.
6. At the UA-6 detector back panel, connect the optical unit cable to the connector labeled OPTICAL UNIT.

Power source connections (Figure 2-2)

7. Locate and connect the AC power adapter shipped with the Tris Pump:
   a. At the Tris pump front panel, connect the 14 VAC connector.

⚠ CAUTION

The AC power source must meet the requirements listed on the AC power adapter. The factory ships Tris pump power adapters for North American or European power outlets. For other locales, it might be necessary to purchase a connector adapter from a local vendor.

   b. Connect the power adapter to an AC power source.
8. The fraction collector and UA-6 detector were shipped with IEC power cords. Use these to connect each instrument to an AC power source.

⚠ CAUTION

Always refer to the instrument's serial number label for mains power requirements.

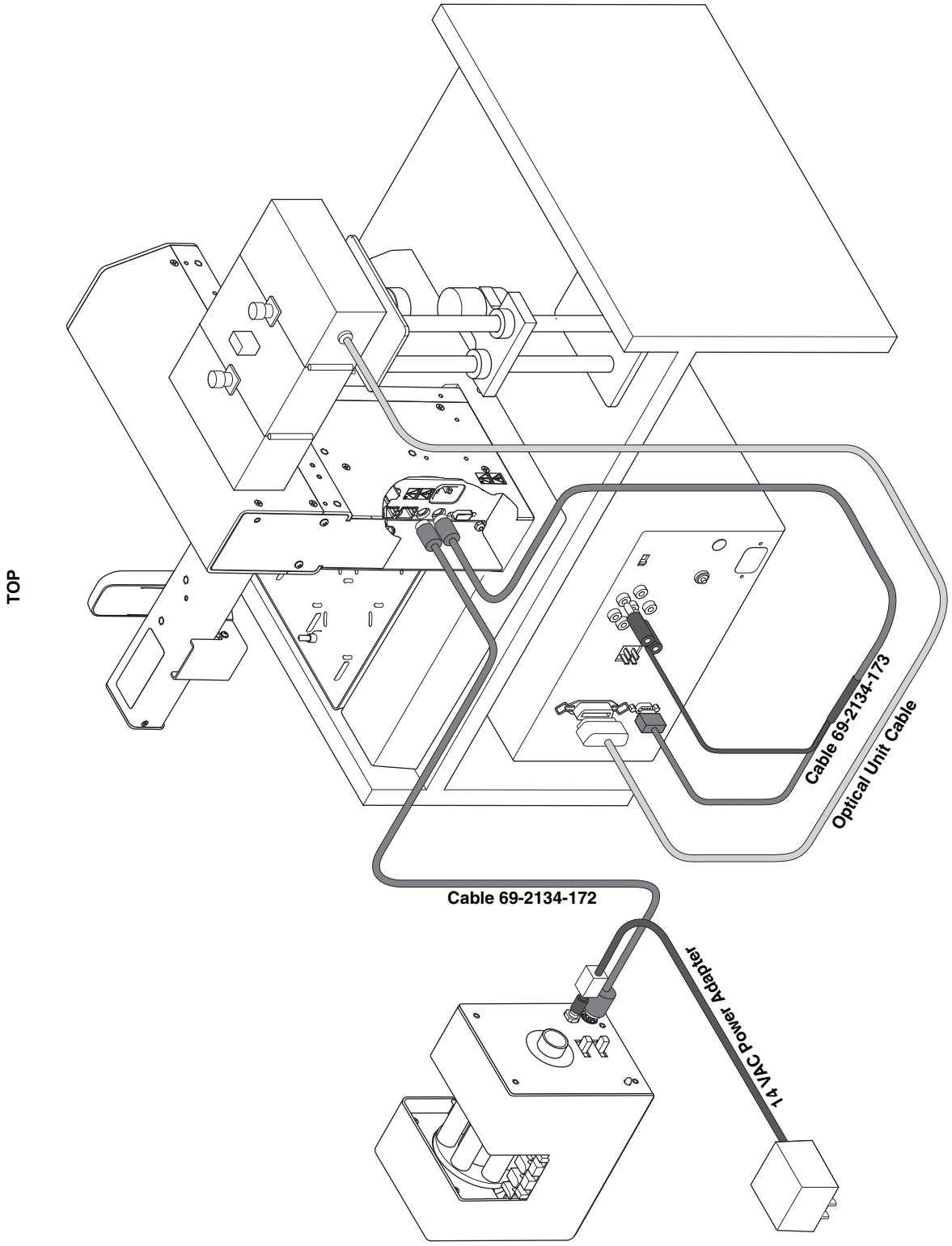


Figure 2-2 System connections with a Foxy R1 fraction collector (rotated view)

⚠ CAUTION

The instruments are shipped with either a North American IEC320C13 to NEMA 5-15P power cord or a European IEC320C13 to CEE7/VII power cord. If the cord does not fit your AC mains power source, purchase a connector adapter or IEC320C13 power cord from a local vendor.

9. Move the Tris pump under the organizer shelf, positioning it towards the front as shown in Figure 1-1 on page 1-2. This will allow you to plumb the system from the front.

System plumbing connections (Figure 2-3)

10. Refer to Figure 2-3, Table 2-1, and Table 2-2 and complete the plumbing connections.

    When using the figure and tables, note the following:

    - Each piece of tubing is identified by an uppercase letter in a circle, such as Ⓐ. Table 2-1 lists the tubing part number and the length you must cut from the bulk tubing supplied in the installation kit.
    - Connection types are identified by numbers in a triangle, such as △1. Table 2-2 lists the hardware pieces from the installation kit and provides instructions to complete the connection.
    - Pieces of the connection hardware are identified by a lowercase letter, such as "a."

☑ *Note*

Only finger-tighten the plumbing fittings. Never use a tool to tighten any system plumbing connection.

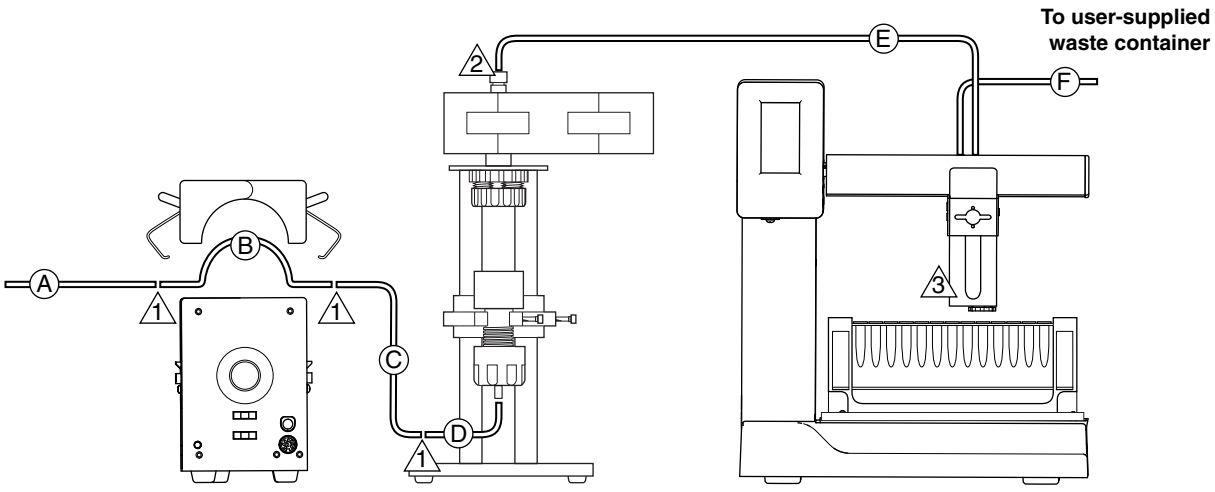
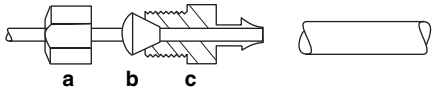
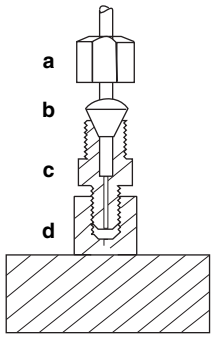
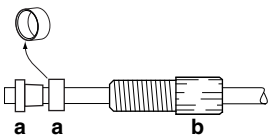


Figure 2-3 System tubing connections with Foxy R1 fraction collector

Table 2-1 Tubing and Lengths

Tubing (Fig. 2-3)	Cut Length	Part Number	Description
Ⓐ	As needed	023-0504-02	PTFE 0.062 ID, 0.125 OD
Ⓑ	8” (200 mm)	029-1304-02 029-1351-06	Silicone 0.125 ID, 0.25 OD, for flow rates greater than 2 mL/minute Silicone 0.065 ID, 0.194 OD, for flow rates less than 2 mL/minute (See Table 3-1 for recommended flow rate ranges.)
Ⓒ	18” (460 mm)	023-0504-02	PTFE 0.062 ID, 0.125 OD
Ⓓ	4” (100 mm)	029-1304-02	Silicone 0.125 ID, 0.25 OD
Ⓔ	21” (530 mm)	023-0504-02	PTFE 0.062 ID, 0.125 OD (tubing volume approximately 1 mL)
Ⓕ	As needed	023-0504-02	PTFE 0.062 ID, 0.125 OD

Table 2-2 Connection Hardware

Union (Fig. 2-3)	Connection Diagram	Item Descriptions and Instructions
△1		a. Connector Nut, 60-0923-015 b. 1/8” Ferrule, 60-0923-017 c. Barbed Connector, 60-1613-112 *Instructions:* 1. Place Ferrule, narrow end first, in Barbed Connector. 2. Loosely thread Connector Nut onto Barbed Connector. 3. Insert PTFE tubing into Connector Nut and finger-tighten. 4. Push silicone tubing over barbed end.
△2		a. Connector Nut, 60-0923-015 b. 1/8” Ferrule, 60-0923-017 c. Black Lead Connector, 60-0923-013 d. Flow cell on Brandel tube piercer *Instructions:* 1. Place Ferrule, narrow end first, in Lead Connector. 2. Loosely thread Connector Nut onto Lead Connector. 3. Insert PTFE tubing into Connector Nut and finger-tighten.
△3		a. Ferrule and Locking Ring, 209-0163-21. Note: Tapered edge of locking ring must face the ferrule. b. Headless Nut, 209-0163-24 *Instructions:* [a] 1. Slide Nut onto PTFE tubing. 2. Slide Locking Ring and Ferrule onto PTFE tubing. Note: Tapered edge of locking ring must face the ferrule. 3. Push PTFE tubing fully into port on back of diverter valve. 4. Finger-tighten Nut to swage Locking Ring and Ferrule.[b]

a. Refer to the Foxy R1 instruction manual for correct routing and identification of inlet and waste ports.

b. An inspection of the swaged fitting is recommended. Un-thread the headless nut and verify that: 1) the yellow ferrule is flush and perpendicular with the end of the tubing; 2) the metal lock ring is compressed over the ferrule without any gaps; and 3) all pieces are correctly aligned and free from any deformation. If not fully swaged, reinsert the headless nut into the port and tighten further. Un-thread the headless nut and inspect again using this criteria.

2.4 Installation of System with Retriever 500 Fraction Collector

1. Position system components on the organizer shelf as shown in Figure 2-4.
2. Ensure that the UA-6 detector power switch is in the OFF position.
3. Set the Tris pump CCW/STOP/CW switch to the STOP position.

Interface cable connections (Figure 2-4)

4. Locate cable 60-1020-217 that was shipped with the system components.
   a. At the back panel of the fraction collector, connect the 8-pin DIN connector to the connector labeled DETECTOR.
   b. At the back panel of the UA-6 detector, connect the 9-pin connector labeled Fraction Collector.
5. Locate cable 60-1020-204 that was shipped with the system components.
   a. At the back panel of the fraction collector, connect the 6-pin DIN connector to the connector labeled PUMP.
   b. At the front panel of the Tris pump, connect the cable to the connector labeled SIGNAL.
6. At the UA-6 detector back panel, connect the optical unit cable to the connector labeled OPTICAL UNIT.

Power source connections (Figure 2-4)

7. Locate and connect the AC power adapter shipped with the Tris Pump:
   a. At the Tris pump front panel, connect the 14 VAC connector.

⚠ CAUTION

The AC power source must meet the requirements listed on the AC power adapter. The factory ships Tris pump power adapters for North American or European power outlets. For other locales, it might be necessary to purchase a connector adapter from a local vendor.

   b. Connect the power adapter to an AC power source.
8. The fraction collector and UA-6 detector were shipped with IEC power cords. Use these to connect each instrument to an AC power source.

⚠ CAUTION

Always refer to the instrument's serial number label for mains power requirements.

⚠ CAUTION

The instruments are shipped with either a North American IEC320C13 to NEMA 5-15P power cord or a European IEC320C13 to CEE7/VII power cord. If the cord does not fit your AC mains power source, purchase a connector adapter or IEC320C13 power cord from a local vendor.

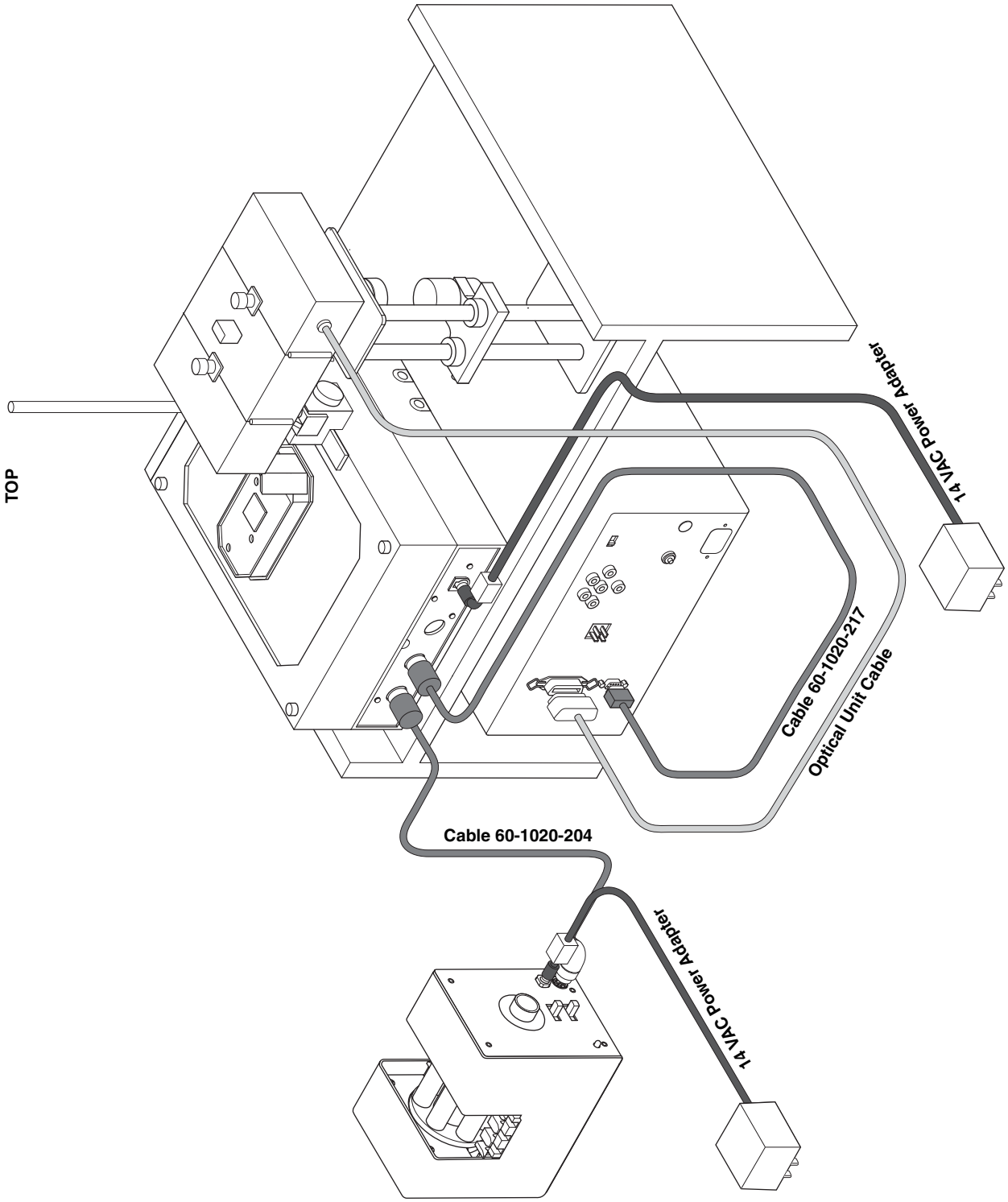


Figure 2-4 System connections with a Retriever 500 fraction collector (rotated view)

9. Move the Tris pump under the organizer shelf, positioning it towards the front as shown in Figure 1-1 on page 1-2. This will allow you to plumb the system from the front.

System plumbing connections (Figure 2-5)

10. Refer to Figure 2-5, Table 2-3, and Table 2-4 and complete the plumbing connections.

    When using the figure and tables, note the following:

    - Each piece of tubing is identified by an uppercase letter in a circle, such as Ⓐ. Table 2-1 lists the tubing part number and the length you must cut from the bulk tubing supplied in the installation kit.
    - Connection types are identified by numbers in a triangle, such as △1. Table 2-4 lists the hardware pieces from the installation kits and provides instructions to complete the connection.
    - Pieces of the connection hardware are identified by a lowercase letter, such as "a."

☑ Note

Only finger-tighten the plumbing fittings. Never use a tool to tighten any system plumbing connection.

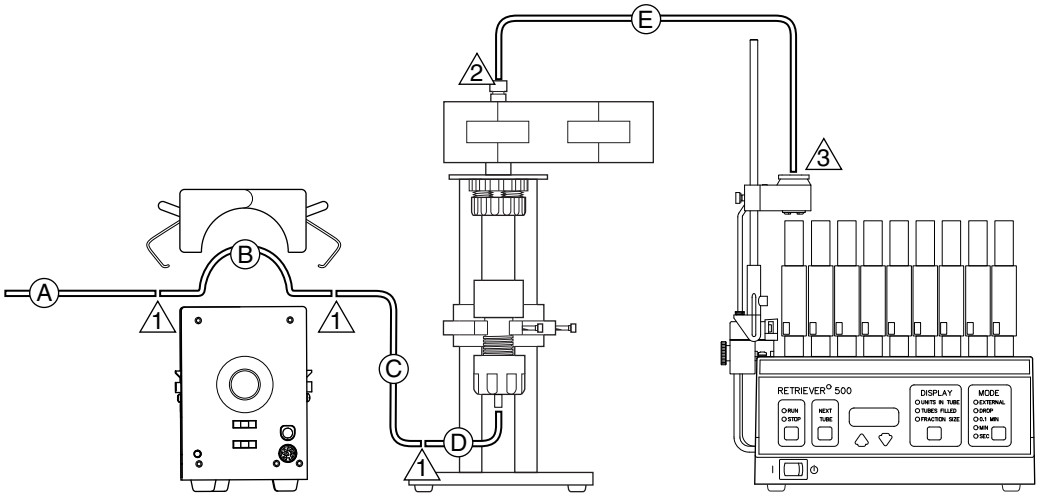
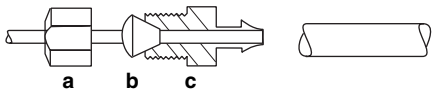
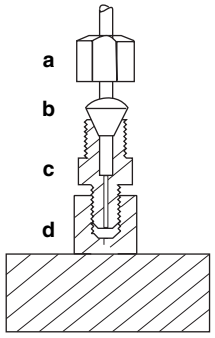
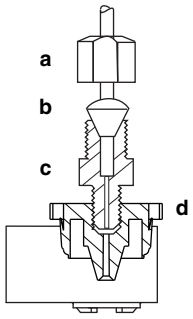


Figure 2-5 System tubing connections with Retriever 500 fraction collector

Table 2-3 Tubing and Lengths

Tubing (Fig. 2-3)	Cut Length	Part Number	Description
Ⓐ	As needed	029-1304-02	Silicone 0.125 ID, 0.25 OD
Ⓑ	8” (200 mm)	029-1304-02 029-1351-06	Silicone 0.125 ID, 0.25 OD, for flow rates greater than 2 mL/minute Silicone 0.065 ID, 0.194 OD, for flow rates less than 2 mL/minute (See Table 3-1 for recommended flow rate ranges.)
Ⓒ	32” (813 mm)	023-0502-04	FEP 0.030 ID, 0.0625 OD
Ⓓ	3” (76 mm)	029-1304-02	Silicone 0.125 ID, 0.25 OD
Ⓔ	16” (406 mm)	023-0502-04	FEP 0.030 ID, 0.0625 OD (tubing volume approximately 0.8 mL)

Table 2-4 Connection Hardware

Union (Fig. 2-3)	Connection Diagram	Item Descriptions and Instructions
△1		a. Connector Nut, 60-0923-015 b. 1/8” Ferrule, 60-0923-017 c. Barbed Connector, 60-1613-112 *Instructions:* 1. Place Ferrule, narrow end first, in Barbed Connector. 2. Loosely thread Connector Nut onto Barbed Connector. 3. Insert PTFE tubing into Connector Nut and finger-tighten. 4. Push silicone tubing over barbed end.
△2		a. Connector Nut, 60-0923-015 b. 1/8” Ferrule, 60-0923-017 c. Black Lead Connector, 60-0923-013 d. Flow cell on Brandel tube piercer *Instructions:* 1. Thread Lead Connector into Flow Cell. 2. Place Ferrule, narrow end first, in Lead Connector. 3. Loosely thread Connector Nut onto Lead Connector. 4. Insert PTFE tubing into Connector Nut and finger-tighten.
△3		a. Connector Nut, 60-0923-015 b. 1/8” Ferrule, 60-0923-017 c. Red Lead Connector, 60-0643-254 d. Drop former on Retriever 500 *Instructions:* 1. Thread Lead Connector into Drop Former. 2. Place Ferrule, narrow end first, in Lead Connector. 3. Loosely thread Connector Nut onto Lead Connector. 4. Insert PTFE tubing into Connector Nut and finger-tighten.

2.5 Changing the Flow Cell Aperture

The flow cell was shipped from the factory with 1.0 mm apertures installed. A larger 2.8 mm aperture set is in the accessory kit should you need to illuminate a larger volume. To change the flow cell apertures:

1. On one side of the flow cell, remove the window nut using the wrench included with the flow cell (Figure 2-6).
2. Remove the 1.0 mm aperture.
3. Verify that the O-ring is in place.
4. Insert the 2.8 mm aperture. Position the aperture so that the opening is horizontal.
5. Install the window nut and tighten.
6. Repeat steps 1 through 5 for the other side.
7. Verify that both light aperture slits are in the horizontal plane as shown in Figure 2-6. If not, loosen the window nuts, align the openings, and tighten the window nuts.

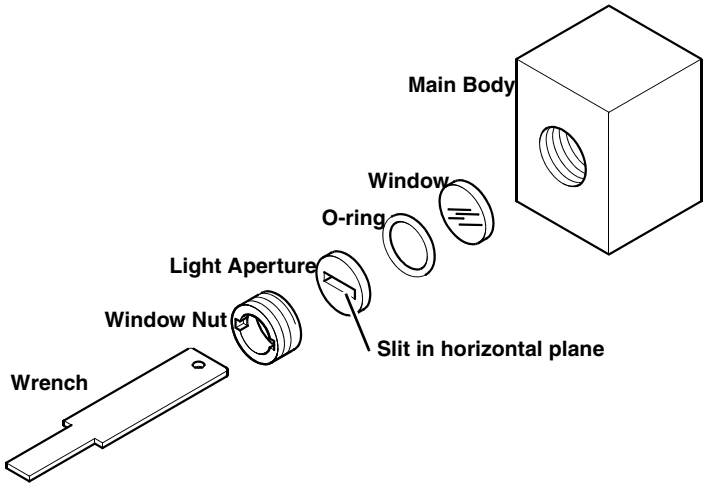


Figure 2-6 Flow cell aperture installation

☑ Note

The illuminated volumes are specified in Table 2-5, as well as the light aperture insert heights and path lengths.

Table 2-5 Flow Cell Illuminated Volumes

Light Aperture Height (mm)	Path Length (mm)	Illuminated Volume (µl)
1.0	2 5	6 14
2.8	2 5	15 39

Density Gradient Fractionation Systems

Section 3 Operation

This section describes the general operation of the Density Gradient Fractionation system. The steps and system settings are intended to serve as the basis for a fractionation method which should be empirically modified for your application. Please refer to the individual component manuals for additional operating instructions.

> ☑ ***Note***
>
> Both models of fraction collectors are discussed in this section. When following instructions relate to fraction collectors perform only those instructions for the installed model.

3.1 System Preparation

This section covers the preliminary system settings for basic fractionation operation and priming the inlet tubing with a chase liquid.

3.1.1 Tris Pump

The maximum flow rate of the chase liquid is limited by a number of factors:

- If a constant speed recorder is used, it may give a compressed scanning curve for high flow rates, resulting in poorer apparent resolution of peaks.
- If a fast flow rate is used with very viscous solutions, pressure may build up in the system and leaks may occur.
- Turbulence or laminar flow may occur at higher flow rates, causing a decrease in resolution observed at the absorbance monitor.
- The recorder pen moves slowly and since the rate of change in optical density will increase with faster speeds, recording inaccuracies and poorer apparent resolution may result.

Teledyne Isco recommends starting at the minimum flow rate range listed in Table 3-1 for the selected centrifuge tube.

0.375 mL/min

To set the flow rate at 0.375 mL/min:

1. Ensure that 0.065 (1/16") ID, 0.194 OD silicone tubing (029-1351-06) is installed in the pump.
2. Place the X1/X10/MAX switch in the X10 position.
3. Set the % CONTROL dial to 15.

1.50 mL/min

To set the flow rate at 1.50 mL/min:

1. Ensure that 0.065 (1/16") ID, 0.194 OD silicone tubing (029-1351-06) is installed in the pump.

2. Place the X1/X10/MAX switch in the X10 position.
3. Set the % CONTROL dial to 60.

3.0 mL/min

To set the flow rate at 3.0 mL/min:

1. Ensure that 0.125 (1/8") ID, 0.25 OD silicone tubing (029-1304-02) is installed in the pump.
2. Place the X1/X10/MAX switch in the X10 position.
3. Set the % CONTROL dial to 36.

☑ Note

Refer to Section 3 of the Tris pump manual for complete information on setting Tris pump flow rates.

Table 3-1 Recommended Flow Rates[a]

Centrifuge Tube Size	Mfg. Rotor Designation	Nominal Flow Rate (mL/min)
7/16 x 1-15/16	Beckman	0.375 to 0.750
7/16 x 2-3/8	Beckman SW 56	
10.9 x 54.7 mm	International SB 405	
1/2 x 2	Beckman SW 39, 50, 65, 50.1	
1/2x 2	Beckman Quick-Seal® 342412	
1/2 x 2-1/2	Beckman Type 40.2 and 40.3	
12.7 x 50.8 mm	International 2865	
12.7 x 98.4 mm	International	
9/16 x 3-1/2	Beckman SW 41	1.50 to 3.0
9/16 x 3-3/4	Beckman SW 40	
14.5 x 96 mm	International SB 283 and 206	
14.5 x 102 mm	International	
5/8 x 2-1/2	Beckman 50	
5/8 x 3	Beckman Type 40 TI-50	
5/8 x 3	Beckman Quick-Seal® 342413	
5/8 x 4	Beckman SW 25.3	
16.1 x 76.2 mm	International 495	
23 x 70 mm	MSE 23 ml	
1 x 3	Beckman SW 25.1	
1 x 3-1/2	Beckman SW 27 and Type 30	3.0 to 6.0
1 x 3-1/2	Beckman Quick-Seal® 342414	
25.4 x 88.9 mm	International SB-110	
1-1/4 x 3-1/2	Beckman SW 25.2	

a. If your centrifuge tube is not listed, refer to a listed tube with the closest dimensions.

3.1.2 UA-6 Detector and Optical Unit

1. Turn the PEAK SEPARATOR knob to the OFF position.
2. Turn the CHART SPEED knob to OFF.
3. Place the STANDBY/OPERATE switch in the OPERATE position.
4. Allow a minimum of 15 minutes warm up time to stabilize the lamp current.
5. Set SENSITIVITY knob to SET LAMP & OPTICS.
6. On the Optical Unit, set the BASELINE ADJUST control to MAX. OPEN.
7. Adjust the BASELINE ADJUST control of the optical unit until the chart pen moves to near zero.

> ☑ ***Note***
>
> If the chart pen does not move to zero, turn the BASELINE ADJUST control of the optical unit to MAXIMUM OPEN. Move SENSITIVITY switch from SET LAMP & OPTICS to desired absorbance range.

8. Align the RECORDER OFFSET control to its top-center mark.
9. Push AUTO BASELINE. The pen should deflect near mid scale.

> ☑ ***Note***
>
> The baseline setting can remain near mid scale or moved to any arbitrary baseline by using the recorder offset control of the UA-6 detector. Positioning the baseline will not alter the accuracy of the reading.

10. Place the NOISE FILTER switch in the 1.5 position.
11. Turn the PEAK SEPARATOR knob to 3.

> ☑ ***Note***
>
> Refer to Section 3 of the UA-6 detector manual for detailed operating information.

3.1.3 Foxy R1 Fraction Collector

At this time you should install a rack filled with tubes on the Foxy R1 fraction collector. Refer to Section 2 of the Foxy R1 and Foxy R2 user manual. After installing the rack, adjust the height of the diverter valve if necessary.

The Foxy R1 fraction collector can now be configured and programmed for operation.

Foxy R1 Configuration

Refer to *Configuration Settings* in Section 3 of the fraction collector user manual and configure the following settings:

1. Touch the display to turn on the fraction collector.
2. Ensure that the RACK 1 setting matches the installed rack and tubes.

3. Set the TUBE ADVANCE SPEED to 2.
4. Set the PUMP CONTROL to 0. This will briefly stop the Tris pump during tube changes.
5. Verify that the ANALOG PEAK setting is at 1000 mV.

Foxy R1 Programming

The fraction collector uses a programmed method to control the fractionation and peak cutting. The method also delays the arm movement so that tube advances occur when material observed by the detector is present at the drop former.

Refer to *Method Settings* in Section 3 of the fraction collector user manual and program the following nominal method which assumes:

- ~1 mL fractions will be collected in 1.5 mL microcentrifuge tubes
- a 21 inch length of PTFE 0.062 inch ID tubing installed between the optical unit and diverter valve (tubing E)
- a 1/16-inch ID tube is in the Tris Pump.

To program the fraction collector:

1. Program a method to collect fractions by volume counts from the Tris pump.
   a. From the main display, touch the FOLDER icon and then the VOLUME icon to fractionate by fixed volumes (Figure 3-1).

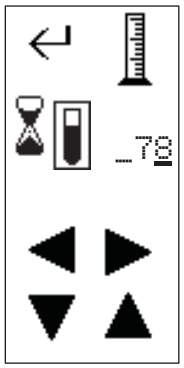


Figure 3-1 Collect fractions by volume counts

   b. Ensure that the TUBE icon is active (highlighted by a surrounding box). If not, touch the icon to toggle the selection.
   c. Enter the volume count as determined by the ID of tubing in the Tris pump.
      With 1/8-inch ID tubing, the pump sends about 22 counts for each milliliter; with 1/16-inch tubing, the pumps sends about 78 counts for each milliliter.
      For this example, enter 78.
   d. Touch the DELAY (hourglass) icon and enter a delay volume of 78 counts. The approximate volume of the tubing from the optical unit to the diverter valve is 1 mL (Table 2-1).

e. Touch the ENTER icon to save the settings and return to the method settings display.

2. Program the method to cut peaks based on the peak width of the analog signal of the UA-6 detector.
   a. Touch the FOLDER icon and then the PEAK WIDTH icon.

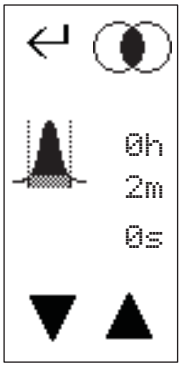


Figure 3-2 Cut peaks by peak widths and collect all fluids

   b. Enter a peak width of 2 minutes.
   c. The peak width icon should be shaded below the peak. This indicates that the fraction collector will collect all fluids instead of diverting non-peak fluids to waste. Touch the icon to toggle the state until the icon is shaded.
   d. Touch the ENTER icon to save the settings and return to the method settings display.
3. From the method settings display, verify that the only the VOLUME and PEAK WIDTH options are active as shown in Figure 3-3. If not, disable the other active features.

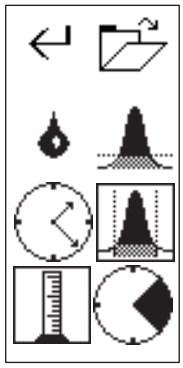


Figure 3-3 Basic method settings

4. Touch the ENTER icon to return to the main display.

The fraction collector is now programmed with a basic collection method. The Foxy R1 fraction collector has many advanced features that may be included in the method such as:

- Combining the PEAK WIDTH with THRESHOLD detection
- Using time windows to divert the initial tubing volume to waste
- Renaming the method

Refer to the user manual for more information.

3.1.4 Retriever 500 Fraction Collector

At this time you should install racks and tubes on the Retriever 500 fraction collector. Refer to Section 2 of the Retriever 500 Fraction Collector R2 user manual. After installing the racks and tubes, adjust the height of the drop counter if necessary.

Refer to *Operating Procedures* in Section 3 of the fraction collector user manual and program the following nominal method which assumes:

- Tubes will be advanced every 78 pump counts (roughly 1 mL)
- a 16 inch length of FEP 0.0625 inch ID tubing installed between the optical unit and diverter valve (tubing E)
- a 1/16-inch ID tube is in the Tris Pump.

To program the fraction collector:

1. Place the POWER switch in the ON (1) position.
2. Push the MODE button to select EXTERNAL.
3. Push the DISPLAY button to select FRACTION SIZE.
4. Push the UP ARROW button to enter volume counts as determined by the ID of tubing in the Tris Pump.
   With 1/8-inch ID tubing, the pump sends about 22 counts for each milliliter; with 1/16-inch tubing, the pumps sends about 78 counts for each milliliter.

   For this example, enter 78.

The fraction collector is ready for operation.

3.1.5 Tube Piercer

The tube piercer uses a septum on the piercing mechanism to secure the bottom of the centrifuge tube. The top assembly uses a ring and collar to secure the top of the tube.

Septum Installation

To install a septum:

1. Turn the bottom cap of the piercing mechanism (Figure 3-4) to lower the piercing needle.
2. Cut a septum from the septum and collar set (Figure 3-5). Use a sharp knife and cleanly trim any excess material from the cut edge.
3. Insert the septum over the spring in the piercing mechanism.

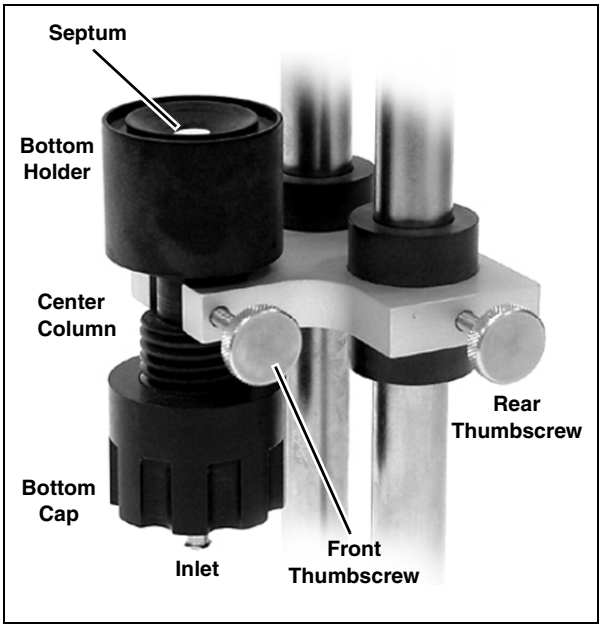


Figure 3-4 Piercing mechanism

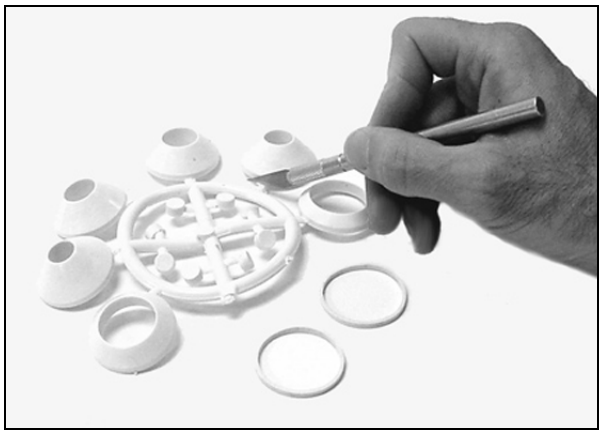


Figure 3-5 Styrene-butadiene rubber septum and collar set

Ring and Collar Installation

A ring and collar secures and seals tubes (≤1 inch diameter) in the top assembly. To install a ring and collar:

1. Determine the size of centrifuge tube and select the collar and ring according to Table 3-2.

☑ Note

If you have selected a 1-1/4 inch diameter tube, skip the ring and collar installation steps. These larger diameter tubes do not use a ring and have an O-ring type collar which is installed at the time the tube is loaded (step 1 note, section 3.2).

Table 3-2 Collar and Ring Selection[a]		
Centrifuge Tube Size	**Mfg. Rotor Designation**	**Collar & Ring Designation**
7/16 x 1-15/16	Beckman	A
7/16 x 2-3/8	Beckman SW 56	A
10.9 x 54.7 mm	International SB 405	A
1/2 x 2	Beckman SW 39, 50, 65, 50.1	B
1/2 x 2-1/2	Beckman Type 40.2 and 40.3	B
12.7 x 50.8 mm	International 2865	B
12.7 x 98.4 mm	International	B
9/16 x 3-1/2	Beckman SW 41	C
9/16 x 3-3/4	Beckman SW 40	C
14.5 x 96 mm	International SB 283 and 206	C
14.5 x 102 mm	International	C
5/8 x 2-1/2	Beckman 50	D
5/8 x 3	Beckman Type 40 TI-50	D
5/8 x 4	Beckman SW 25.3	D
16.1 x 76.2 mm	International 495	D
23 x 70 mm	MSE 23 ml	F Collar / E Ring
1 x 3	Beckman SW 25.1	E
1 x 3-1/2	Beckman SW 27 and Type 30	E
25.4 x 88.9 mm	International SB-110	E
1-1/4 x 3-1/2	Beckman SW 25.2	—
1/2x 2	Beckman Quick-Seal® 342412	G Collar / F Ring
5/8 x 3	Beckman Quick-Seal® 342413	G
1 x 3-1/2	Beckman Quick-Seal® 342414	H

a. If your centrifuge tube is not listed, simply select the best fitting collar.

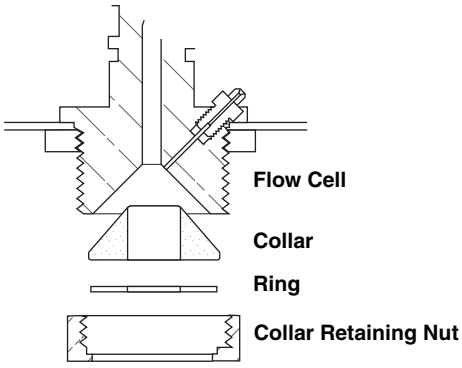


Figure 3-6 Install the collar and ring

2. Cut the collar from the septum and collar set (Figure 3-5). Use a sharp knife and trim excess material from the cut edge.
3. Remove the collar retaining nut from the piercing mechanism Figure 3-6.
4. Insert the collar into the flow cell.
5. Insert the stainless steel ring into the groove inside the collar retaining nut.
6. Loosely screw the collar retaining nut onto the flow cell to hold the collar and ring in place.

3.1.6 Chase Liquid

Fractionation is performed by pumping a chase liquid through the system. The chase liquid gently pushes the contents of the centrifuge tube through the UV detector, then into the fraction collection tubes.

Typically, chase liquids are prepared just as the gradient solution, except that the chase liquid must be a higher concentration (*i.e.* more dense). See Appendix A for tables of chase liquids. Adding dye to the chase liquid is a convenient method to ensure that the entire contents of the centrifuge tube have been delivered to the fraction collector.

The chase liquid must be more dense than the solution at the bottom of the gradient in the centrifuge tube. Fluorinert FC-40 electronic liquid (P/N 68-0647-021) is a very satisfactory chase liquid for all common gradient materials. Sucrose solutions are widely used as a chase liquid; however, sucrose solutions more concentrated than 1.8M (620 g/L) are too viscous to be forced through the small orifices of the system and should not be used.

Sucrose solutions may be used to chase sucrose, glycerine, Ficoll, or dextran gradients, but cannot be successfully used alone to chase dense solutions of salts such as NaBr or CsCl, nor can the salts be used to chase sucrose. Convection and disruption of the bottom of the gradient column occur if sugar solutions are chased with salt solutions or vice versa. This convection is apparently a result of the widely different diffusion rates of salt and sucrose and the resulting loss of salt from the salt solution next to the sucrose solution. The use of Fluorinert FC-40 is highly recommended in all cases.

After selecting a chase liquid:

1. Prepare more than enough chase liquid to fill the centrifuge tube volume as well as the volume of system tubing. This will ensure that the pump will deliver the full gradient to the fraction collector and leave the system primed for the next run.
2. Place the Tris pump's inlet tubing into the chase liquid container.
3. Place an absorbent wipe over the Tube Piercer needle opening.

4. Pump chase liquid until the liquid just reaches the Tube Piercer needle opening. To do so:
   a. Place the CCW/OFF/CW switch in the CW position. The pump begins to operate. Observe the flow of chase liquid through the pump and up to the Tube Piercer.
   b. When the chase liquid reaches the needle opening, place the CCW/OFF/CW switch in the OFF position.

☑ Note

If there is air in the tubing, allow the pump to continue to run to clear the air bubbles. Air bubbles may disturb the material suspended in the gradient and contribute noise to peak detection.

3.2 Fractionation

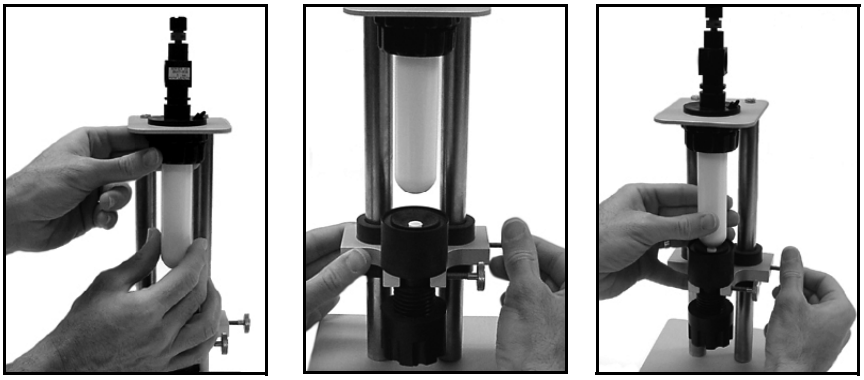
To fractionate a density gradient centrifuge tube:

1. Insert a tube (≤1 inch diameter) into the Tube Piercer:
   a. Loosen the rear thumbscrew and lower the tube piercing mechanism.
   b. Fully insert the tube into the top assembly through the collar retaining nut (Figure 3-7).
   c. Hand-tighten the collar retaining nut to seal the tube.

⚠ CAUTION

Do not over-tighten the nut. Over-tightening the nut may deform the tube wall or tear the collar resulting in leaks. The nut should be only snug.

   d. Ensure that the tube is vertically aligned. If not, push the tube to align it.
   e. Raise the bottom tube piercing mechanism until the septum is depressed by about 5 mm ($^3/_{16}$-inch) against the spring.
   f. Tighten the thumbscrew to secure the tube.



1. Insert tube and tighten collar retaining nut. **2. Loosen rear thumbscrew.** **3. Raise the tube piercing mechanism and tighten thumbscrew.**

Figure 3-7 Inserting the centrifuge tube

☑ Note

If you are fractionating a 1-1/4 inch diameter tube, place the O-ring seal around the top edge of the tube. Next, remove the collar retaining nut and insert the tube so it is centered in the assembly. Replace the collar retaining nut and hand-tighten to seal the tube. Then, raise the tube piercing mechanism and tighten the thumbscrew.

2. Pierce the tube.

⚠ CAUTION

Tubes made of materials such as polycarbonate and glass cannot be pierced.

a. Turn the bottom cap to raise the needle (Figure 3-8).

b. Continue to turn the bottom cap while watching the bottom of the tube. The tip of the needle will pierce the tube.

c. Stop turning the bottom cap when the holes in the needle tip are just inside the tube.

Figure 3-8 Piercing the centrifuge tube

3. Start the UA-6 detector:

a. Uncap the pen.

b. Select the SENSITIVITY. This setting should be the expected absorbance. If unknown, use 0.2 as an initial value.

c. Adjust the RECORDER OFFSET for the desired pen position.

d. Select the desired CHART SPEED. A setting of 15 cm/hr is a suggested nominal value. Refer to the UA-6 and Tris pump manuals for more information about chart speed.

4. At the Tris pump, place the CCW/OFF/CW switch in the CW position. The chase liquid will begin filling the centrifuge tube.

5. Start the fraction collector.
   - Foxy R1: touch the PLAY icon.
   - Retriever 500: push the RUN/STOP button.

The system will begin to fractionate the centrifuge tube contents. During operation you can observe the following:

Chase liquid – If a dye was added to the liquid, you can observe the dense chase liquid displacing the gradient.

Fractionation – The fraction collector will perform a tube advance at the set fractionation interval. Using these nominal program settings, the fraction collector will change tubes every 1 mL (approximate).

Peak detection – The UA-6 detector charts the absorbance. If peaks exceed the chart scale, adjust the sensitivity as needed.

An LED indicator on the front panel also indicates the current peak state:

- The LED is off as the chart continues along the baseline (zero slope).
- During a positive slope, the LED is green.
- At the maximum of the peak (zero slope) the LED is off.
- As the slope returns toward the baseline with downward slope, the LED is red.
- The LED turns off when the slope returns to zero. (This might not be at the original baseline.)

Peak cutting – The fraction collector will perform a tube advance at the beginning and end of a peak to isolate peak materials from non-peak. The Retriever 500 will perform these tube advances when the LED on the UA-6 changes from off to green and when it changes from red to off. The Foxy R1 was programmed for internal peak detection with a delay volume. Because the peaks are being cut much closer to when the material is present at the drop former, these tube advances will not coincide with the LED on the UA-6 detector.

Stopping the Fractionation Run

Stop the run when the all of the contents of the density gradient tube have been pushed to the fraction collector:

1. At the Tris pump, place the CCW/OFF/CW switch in the OFF position.
2. Stop the fraction collector.
   - Foxy R1: touch the STOP icon.
   - Retriever 500: push the RUN/STOP button.
3. At the UA-6 detector, set the CHART SPEED to OFF.

3.3 Post Fractionation

After stopping the fractionation run, the fractions may be removed from the fraction collector.

The following steps should be performed to remove the centrifuge tube and prepare the system for the next fractionation run.

1. At the Tris pump, place the CCW/OFF/CW switch in the CCW position. This removes the chase liquid from the tubing.

☑ *Note*

The Tris pump % CONTROL knob may be turned to 100% to expedite this step.

2. When the chase liquid has withdrawn to a point just above the piercing needle place the CCW/OFF/CW switch in the OFF position. This small volume above the needle ensures that air will not be in the needle for the next run.

☑ *Note*

If the Tris pump % CONTROL knob was turned to 100%, return it to the original setting now.

3. Loosen the rear thumbscrew and lower the tube piercing mechanism.
4. Loosen the collar retaining nut.
5. Remove the centrifuge tube from the tube piercer. Clean up excess chase liquid.
6. Load the fraction collector with empty tubes.
7. Very small amounts of chase liquid will be lost at the end of the run and when the centrifuge tube is removed. Replenish the chase liquid container if necessary (Section 3.1.6).
8. Review the operation settings of the pump, detector and fraction collector. Revise the settings as necessary to optimize the system for your application.

The system is ready for the next fractionation run. Refer to Section 3.2.

3.4 Post Run Cleaning

The system should be cleaned when you are finished with all fractionation runs for the day. This will prevent material from collecting or crystallizing in the liquid path.

1. Remove the chase liquid container. Cap and store if desired.
2. Remove all tubing.
3. Remove the optical unit from the top of the tube piercer.

⚠ CAUTION

Do not immerse any electronic instrument component.

4. Remove the flow cell and upper assembly from the tube piercer stand. Separate the flow cell from the upper assembly.
5. Remove the bottom tube piercing mechanism from the tube piercer stand. This is held in place by the front thumbscrew.
6. Wash the tubing, tubing connectors, flow cell, upper assembly, tube piercing mechanism with needle. Generally hot water and a mild detergent is sufficient.

☑ Note

The flow cell windows do not normally need to be removed for cleaning.

☑ Note

If the piercing needle becomes clogged, it may be cleaned with the piece of 22 gauge (0.029 inch diameter) wire supplied with the tube piercer.

7. Clean any spillage of solution on the instruments and shelf.
8. Reassemble the system when the parts are clean and dry.

Density Gradient Fractionation System

Appendix A Tables

A.1 Density Gradient Tables

The following tables are applicable to typical Density Gradient Fractionation applications:

- Table A-1, *Viscosity of various salt solutions used for density gradients*
- Table A-2, *Density at 25 °C of various solutions used for density gradients*
- Table A-3, *Density of aqueous sucrose solutions, g/mL*
- Table A-4, *Centrifugal force chart for rotors commonly used in density gradient centrifugation*
- Table A-5, *Sedimentation rates and ultraviolet absorbances of some representative viruses*

Table A-1 Viscosity of various salt solutions used for density gradients [a]

Solute	Temp (°C)	Relative viscosity for a molal concentration of							
		0.5	1	2	3	4	5	10	15
LiCl	0	1.069	1.129		1.454				
	25	1.069	1.142	1.302	1.479	1.673	1.895	3.73	8.23
KBr	0		0.913	0.845	0.817				
	25	0.984	0.969	0.967	1.007				
NaBr	25	1.029	1.062	1.154					
RbBr	25	0.979							
CsCl	25		0.985	0.975					
Cs_2SO_4	25	1.067	1.145						
K acetate	18	1.125	1.248	1.515	1.817	2.172			
K tartrate [b]	18	1.183							

a. Values selected from *International Critical Tables*, and Landolt-Bornstein, *Zahlen und Funktionen aus Physik, Chemie, Geophysik und Technik*, 7th Ed.

b. 1.74 at 1.5 molal.

Table A-2 Density at 25 °C of various solutions used for density gradients [a]

Solute	Concentration, wt. %					
	10	20	30	40	50	60
LiCl	1.054	1.113	1.178	1.250		
LiBr	1.073	1.160	1.261	1.381	1.53	1.716
KBr	1.072	1.158	1.257	1.371		
NaBr	1.078	1.172	1.281	1.410		
RbBr	1.079	1.174	1.285	1.419	1.582	
CsCl [b]	1.079	1.174	1.286	1.420	1.582	1.785
CsBr	1.081	1.180	1.297	1.440	1.616	
Cs_2SO_4 [b]	1.086	1.190				
K acetate	1.048	1.100	1.155	1.213	1.272	1.333
K citrate	1.066	1.140	1.221			
K tartrate	1.066	1.139	1.218	1.305	1.400	
glycerol	1.021	1.045	1.071	1.097	1.124	1.151
sucrose [c]	1.0381	1.081	1.127	1.176	1.230	1.289
Metrizamide [d]		1.108		1.218		1.328
Fluorinert FC48 [e]	— 1.93 g/mL at 25 °C —					

a. Values are from *International Critical Tables*. Highest values given are not necessarily for saturated solutions.

b. The density of CsCl solutions may be calculated from the formula wt. % = 137.48 – 138.11 ($1\ \rho_4^{25}$) for 30–60% solutions. Data from J. Vinograd and J.E. Hearst, *Progress in the Chemistry of Organic Natural Products XXI*, L. Zechmeister ed., Springer (1962).

The density of Cs_2SO_4 solutions may be calculated from the following formula: $\rho_{25} = 1.0047 + 0.28369m - 0.017428m^2$ ($0.5 \leq m \leq 3.5$) where m is the molality (Ludlum and Warner, *J. Biol. Chem.* **240**, 2961 [1965]).

c. Specific gravity 20°/ 4°C.

d. Density at 15 °C. Metrizamide is a trademark of Nyegaard & Co.

e. Reg T.M. 3M Co.

Table A-3 Density of aqueous sucrose solutions, g/mL [a]

Fraction Sucrose by Weight	g/mL Solution	Temperature (°C)															
		0.0	2.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0	18.0	20.0	22.0	24.0	26.0	28.0	30.0
0.00		1.0004	1.0004	1.0004	1.0004	1.0003	1.0002	1.0000	0.9998	0.9995	0.9992	0.9988	0.9984	0.9980	0.9974	0.9969	0.9963
0.01	10.02	1.0043	1.0043	1.0043	1.0043	1.0041	1.0040	1.0038	1.0036	1.0033	1.0030	1.0026	1.0022	1.0017	1.0012	1.0006	1.0000
0.02	20.12	1.0082	1.0082	1.0082	1.0081	1.0080	1.0079	1.0076	1.0074	1.0071	1.0068	1.0064	1.0059	1.0055	1.0049	1.0044	1.0037
0.03	30.30	1.0122	1.0122	1.0122	1.0121	1.0119	1.0118	1.0115	1.0113	1.0109	1.0106	1.0102	1.0097	1.0093	1.0087	1.0081	1.0075
0.04	40.56	1.0162	1.0162	1.0161	1.0160	1.0159	1.0157	1.0154	1.0152	1.0148	1.0145	1.0140	1.0136	1.0131	1.0126	1.0120	1.0113
0.05	50.89	1.0203	1.0202	1.0202	1.0200	1.0199	1.0196	1.0194	1.0191	1.0187	1.0184	1.0179	1.0175	1.0170	1.0164	1.0158	1.0152
0.06	61.31	1.0244	1.0243	1.0242	1.0241	1.0239	1.0236	1.0234	1.0231	1.0227	1.0223	1.0219	1.0214	1.0209	1.0203	1.0197	1.0191
0.07	71.81	1.0285	1.0284	1.0283	1.0281	1.0277	1.0274	1.0271	1.0263	1.0258	1.0254	1.0267	1.0254	1.0248	1.0243	1.0237	1.0230
0.08	82.40	1.0326	1.0325	1.0324	1.0322	1.0320	1.0317	1.0314	1.0311	1.0307	1.0303	1.0298	1.0293	1.0288	1.0282	1.0276	1.0270
0.09	93.60	1.0368	1.0367	1.0366	1.0361	1.0358	1.0355	1.0352	1.0348	1.0343	1.0339	1.0339	1.0334	1.0328	1.0322	1.0316	1.0310
0.10	103.80	1.0411	1.0409	1.0407	1.0405	1.0403	1.0400	1.0396	1.0393	1.0389	1.0384	1.0380	1.0374	1.0369	1.0363	1.0357	1.0350
0.11	114.70	1.0453	1.0452	1.0450	1.0447	1.0445	1.0441	1.0438	1.0434	1.0430	1.0426	1.0421	1.0415	1.0410	1.0404	1.0397	1.0391
0.12	125.60	1.0496	1.0494	1.0492	1.0490	1.0487	1.0484	1.0480	1.0476	1.0472	1.0467	1.0462	1.0457	1.0451	1.0445	1.0438	1.0432
0.13	136.60	1.0539	1.0537	1.0535	1.0532	1.0529	1.0526	1.0522	1.0518	1.0514	1.0509	1.0504	1.0498	1.0492	1.0486	1.0480	1.0473
0.14	147.70	1.0583	1.0581	1.0578	1.0575	1.0572	1.0569	1.0565	1.0561	1.0556	1.0551	1.0546	1.0540	1.0534	1.0528	1.0522	1.0515
0.15	158.90	1.0627	1.0625	1.0622	1.0619	1.0616	1.0612	1.0608	1.0603	1.0599	1.0594	1.0588	1.0583	1.0577	1.0570	1.0564	1.0557
0.16	170.20	1.0671	1.0669	1.0666	1.0663	1.0659	1.0655	1.0651	1.0647	1.0642	1.0637	1.0631	1.0626	1.0619	1.0613	1.0606	1.0599
0.17	181.50	1.0716	1.0713	1.0710	1.0707	1.0703	1.0699	1.0695	1.0690	1.0685	1.0680	1.0675	1.0669	1.0662	1.0656	1.0649	1.0642
0.18	193.00	1.0761	1.0758	1.0755	1.0751	1.0747	1.0743	1.0739	1.0734	1.0729	1.0714	1.0718	1.0712	1.0706	1.0699	1.0692	1.0685
0.19	204.50	1.0806	1.0803	1.0800	1.0796	1.0792	1.0788	1.0783	1.0778	1.0773	1.0768	1.0762	1.0756	1.0749	1.0743	1.0736	1.0728
0.20	216.20	1.0852	1.0849	1.0845	1.0841	1.0837	1.0833	1.0828	1.0823	1.0818	1.0812	1.0806	1.0800	1.0794	1.0787	1.0780	1.0772
0.21	227.90	1.0898	1.0894	1.0891	1.0887	1.0882	1.0878	1.0873	1.0868	1.0863	1.0857	1.0851	1.0845	1.0838	1.0831	1.0824	1.0816
0.22	239.80	1.0944	1.0940	1.0937	1.0933	1.0928	1.0923	1.0919	1.0913	1.0908	1.0902	1.0896	1.0889	1.0883	1.0876	1.0869	1.0861
0.23	251.70	1.0991	1.0987	1.0983	1.0979	1.0974	1.0969	1.0964	1.0959	1.0953	1.0947	1.0941	1.0935	1.0928	1.0921	1.0914	1.0906
0.24	263.80	1.1038	1.1034	1.1030	1.1025	1.1021	1.1016	1.1010	1.1005	1.0999	1.0993	1.0987	1.0980	1.0973	1.0966	1.0959	1.0951
0.25	275.90	1.1085	1.1081	1.1077	1.1072	1.1067	1.1062	1.1057	1.1051	1.1045	1.1039	1.1033	1.1026	1.1019	1.1012	1.1005	1.0997
0.26	288.10	1.1133	1.1129	1.1124	1.1119	1.1114	1.1109	1.1104	1.1098	1.1092	1.1086	1.1079	1.1072	1.1065	1.1058	1.1051	1.1043
0.27	300.50	1.1181	1.1176	1.1172	1.1167	1.1162	1.1157	1.1151	1.1145	1.1139	1.1133	1.1126	1.1119	1.1112	1.1105	1.1097	1.1089
0.28	312.90	1.1129	1.1225	1.1220	1.1215	1.1210	1.1204	1.1198	1.1192	1.1186	1.1180	1.1173	1.1166	1.1159	1.1151	1.1144	1.1136
0.29	325.40	1.1278	1.1273	1.1268	1.1263	1.1258	1.1252	1.1246	1.1240	1.1234	1.1227	1.1221	1.1213	1.1206	1.1199	1.1191	1.1183
0.30	338.10	1.1327	1.1322	1.1317	1.1312	1.1306	1.1301	1.1295	1.1288	1.1282	1.1275	1.1268	1.1261	1.1254	1.1246	1.1238	1.1230
0.31	350.80	1.1376	1.1371	1.1366	1.1361	1.1355	1.1349	1.1343	1.1337	1.330	1.1323	1.1316	1.1309	1.1302	1.1294	1.1286	1.1278
0.32	363.70	1.1426	1.1421	1.1416	1.1410	1.1404	1.1398	1.1392	1.1386	1.1379	1.1372	1.1365	1.1358	1.1350	1.1342	1.1334	1.1326
0.33	376.60	1.1476	1.1471	1.1466	1.1460	1.1454	1.1448	1.1441	1.1435	1.1428	1.1421	1.1414	1.1406	1.1399	1.1391	1.1383	1.1374
0.34	389.80	1.1527	1.1521	1.1516	1.1510	1.1504	1.1498	1.1491	1.1484	1.1477	1.1470	1.1463	1.1455	1.1448	1.1440	1.1432	1.1423
0.35	402.90	1.1578	1.1572	1.1566	1.1560	1.1554	1.1548	1.1541	1.1534	1.1527	1.1520	1.1513	1.1505	1.1497	1.1439	1.1481	1.1472
0.36	416.20	1.1629	1.1623	1.1617	1.1611	1.1605	1.1598	1.1591	1.1585	1.1577	1.1570	1.1563	1.1555	1.1547	1.1539	1.1530	1.1522
0.37	429.70	1.1680	1.1674	1.1668	1.1662	1.1656	1.0649	1.1642	1.1635	1.1628	1.1620	1.1613	1.1605	1.1597	1.1589	1.1580	1.1572
0.38	443.20	1.1732	1.1726	1.1720	1.1713	1.1707	1.1700	1.1693	1.1686	1.1679	1.1671	1.1663	1.1656	1.1647	1.1639	1.1631	1.1622
0.39	456.80	1.1784	1.1778	1.1772	1.1765	1.1758	1.1752	1.1748	1.1737	1.1730	1.1722	1.1714	1.1706	1.1698	1.1690	1.1681	1.1672
0.40	470.60	1.1837	1.1830	1.1824	1.1817	1.1810	1.1803	1.1796	1.1789	1.1781	1.1774	1.1766	1.1758	1.1749	1.1741	1.1732	1.1723
0.41	484.50	1.1889	1.1883	1.1876	1.1870	1.1863	1.1856	1.1848	1.1841	1.1833	1.1825	1.1817	1.1809	1.1801	1.1792	1.1784	1.1775
0.42	498.40	1.1943	1.1936	1.1929	1.1922	1.1915	1.1908	1.0901	1.1893	1.1886	1.1878	1.1870	1.1861	1.1853	1.1844	1.1835	1.1826
0.43	512.60	1.1996	1.1989	1.1983	1.1976	1.1968	1.1961	1.1954	1.1946	1.1938	1.1930	1.1922	1.1914	1.1905	1.1896	1.1887	1.1878
0.44	526.80	1.2050	1.2043	1.2036	1.2029	1.2022	1.2014	1.2007	1.1999	1.1991	1.1983	1.1975	1.1966	1.1958	1.1949	1.1940	1.1931
0.45	541.10	1.2104	1.2097	1.2090	1.2083	1.2076	1.2068	1.2060	1.2052	1.2044	1.2036	1.2028	1.2019	1.2010	1.2002	1.1993	1.1983
0.46	555.60	1.2159	1.2152	1.2144	1.2137	1.2130	1.2122	1.2114	1.2106	1.2098	1.2090	1.2081	1.2073	1.2064	1.2055	1.2046	1.2036
0.47	570.20	1.2214	1.2206	1.2199	1.2192	1.2184	1.2176	1.2168	1.2160	1.2152	1.2144	1.2135	1.2126	1.2117	1.2108	1.2099	1.2090
0.48	584.90	1.2269	1.2262	1.2254	1.2247	1.2239	1.2231	1.2223	1.2215	1.2206	1.2198	1.2189	1.2180	1.2171	1.2162	1.2153	1.2144
0.49	599.80	1.2325	1.2318	1.2310	1.2302	1.2294	1.2286	1.2278	1.2270	1.2261	1.2253	1.2243	1.2234	1.2225	1.2216	1.2207	1.2198
0.50	614.80	1.2381	1.2374	1.2365	1.2358	1.2349	1.2341	1.2333	1.2325	1.2316	1.2308	1.2298	1.2288	1.2279	1.2270	1.2261	1.2252

a. Calculated from equations developed by E.J. Barber in *National Cancer Institute Monograph*, **21**, June (1966).

Table A-4 Centrifugal force chart for rotors commonly used in density gradient centrifugation [a]

Rotor Mfg and #	Beckman SW 25.1	Beckman SW 25.2	IEC SB-110	Beckman SW 25.3	Beckman SW 27	Beckman SW 27	IEC SB-206 SB-283	Beckman SW 40	Beckman SW 41	Beckman SW-39L SW-50L	IEC SB-405	Beckman SW 65L	Sorvall A-841 T-865
Tube Size diameter: length:	1" 3"	1-1/4" 3-1/2"	25.4 88.9 mm	5/8" 4"	5/8" 4"	1" 3-1/2"	14.5 96 mm	9/16" 3-3/4"	9/16" 3-1/2"	1/2" 2"	10.9 54.7 mm	1/2" 2"	1" 3-1/2"
RPM													
2,500	901	1,069	1,098	1,131	1,160	1,125	1,051	1,111	1,062	685	704	621	635
5,000	3,606	4,276	4,394	4,525	4,640	4,500	4,203	4,444	4,249	2,739	2,817	2,488	2,540
7,500	8,112	9,622	9,886	10,181	10,440	10,125	9,457	9,999	9,559	6,163	6,339	5,597	5,716
10,000	14,422	17,105	17,575	18,100	18,560	18,000	16,812	17,778	16,994	10,956	11,269	9,950	10,160
12,500	22,541	26,735	27,470	28,290	29,000	28,134	26,277	27,784	26,562	17,124	17,613	15,552	15,878
15,000	32,449	38,486	39,544	40,725	41,760	40,500	37,827	39,996	38,237	24,651	25,355	22,388	22,865
17,500	44,174	52,393	53,832	55,440	56,840	55,134	51,495	54,449	52,053	33,558	34,517	30,477	31,121
20,000	57,688	68,420	70,300	72,400	74,240	72,000	67,248	71,105	67,976	43,824	45,076	39,801	40,648
22,500	73,018	86,603	88,982	91,640	93,960	91,134	85,119	90,001	86,041	55,470	57,055	50,378	51,445
25,000	90,131[b]	106,906[b]	109,844[b]	113,125[b]	116,000	112,500	105,075	111,101	106,213	68,475	70,431	62,189	63,512
27,500					140,360[b]	136,134[b]	127,149	134,441	128,526	82,860	85,227	75,253	76,850
30,000							151,308	159,986	152,946	98,604	101,421	89,552	91,443
32,500							177,535	187,717	179,456	115,695	119,001	105,074	107,336
35,000							205,947[c]	217,758	208,177	134,211	138,045	121,890	124,485
37,500							236,377	249,933	238,936	154,041	158,442	139,900	142,903
40,000							268,992[d]	284,419[b]	271,904[b]	175,396[d]	180,304	159,203	162,592[e]
42,500										197,893	203,518	179,725	183,551
45,000										221,867	228,197	201,492	205,781
47,500										247,195	254,229	224,506	229,280
50,000										273,910[f]	281,725	248,755	254,009
52,500											310,574	274,257	280,090
55,000											340,887	300,994	307,400
57,500											372,553	328,536	335,981
60,000											405,684[b]	258,207	365,832
62,500												388,685	396,953
65,000												420,396[b]	429,275[g]

a. Values given are for Maximum Radius of Rotor.
b. Maximum recommended speed for this rotor.
c. Maximum recommended speed for SB-283 and SW-39 rotors.
d. Maximum recommended speed for SW-50L rotor.
e. Maximum for A-841 rotor is 41,000 RPM.
f. Maximum recommended speed for SW-50L rotor.
g. Maximum for T-865 rotor.

Table A-5 Sedimentation rates and ultraviolet absorbances of some representative viruses [a]

Virus	S^{W}_{20}	Absorbance [1mg/mL] – 1 cm at 260 nm	Reference
Alfalfa mosaic	Tz 53, To 60,Ta 68, Tb 76, M 89, B 99	5.2	Bos, L. & Jaspers, E.M.J., *C.M.I./A.A.B.* **46**, June (1971)
Apple chlorotic leaf spot	96		Lister, R.M., *C.M.I./A.A.B.* **30**, Oct. (1970)
Apple mosaic	88, 117		Fulton, R.W., *C.M.I./A.A.B.* **83**, June (1972)
Arabis Mosaic	129		Harrison & Nixon, *Virology* **12**, 104 (1960)
Barley stripe mosaic	185	2.6	Atebekov, J.G., & Novikov, V.K., *C.M.I./A.A.B.* **68**, Oct. (1971)
Barley yellow dwarf	117		Rochow & Brakke, *Virology* **24**, 310 (1964)
Bean pod mottle	54, 91, 112	8.7	Semancek, J.S., *C.M.I./A.A.B.* **108**, Oct. (1972)
Belladonna	53, 113		Paul, H.L., *C.M.I./A.A.B.* **52**, June (1971)
Black raspberry latent	81, 89, 98		Lister & Converse, *C.M.I./A.A.B.* **106**, Oct. (1972)
Broad bean mottle	84.8		Gibbs, A.J., *C.M.I./A.A.B.* **101**, Oct. (1972)
Broad bean stain	60, 100, 127		Gibbs & Smith, *C.M.I./A.A.B.* **29**, Oct. (1970)
Broad bean true mosaic	98, 119		Paul, H.L., *C.M.I./A.A.B.* **20**, June (1970)
Broad bean wilt	63, 100, 126		Taylor & Stubbs, *C.M.I./A.A.B.* **81**, June (1972)
Broccoli necrotic yellows	874 ± 41		Campbell & Lin, *C.M.I./A.A.B.* **85**, June (1972)
Brome mosaic	86	4.8	Bockstahler & Kaesburg, *J. Biophys* **2**, 1 (1962) Brakke, M.K., unpublished data
Cacao swollen shoot	218		Brunt, A.A., *C.M.I./A.A.B.* **10**, June (1970)
Cacao yellow mosaic	49, 108		Brunt, A.A., *C.M.I./A.A.B.* **11**, June (1970)
Carnation latent	167		Paul & Welton, *Phytopath. Z.* **49**, 401 (1964)
Carnation mottle	122		Hollings & Stone, *C.M.I./A.A.B.* **7**, June (1970)
Carnation ringspot	135		Hollings & Stone, *C.M.I./A.A.B.* **21**, Oct. (1970)
Carnation vein mottle	144		Hollings & Stone, O.W., *C.M.I./A.A.B.* **78**, Oct. (1971)
Cauliflower mosaic	220		Shepherd, R.M., *C.M.I./A.A.B.* **24**, Oct. (1970)
Cherry leaf roll	114, 132		Cropley & Tomlinson, *C.M.I./A.A.B.* **80**, Oct. (1971)
Chrysanthemum virus B	168		Hollings & Stone, *C.M.I./A.A.B.* **110**, Oct. (1972)
Citrus leaf rugose virus	79, 89, 98, 105		Garnsey, C.M., Gonsalves, D., *C.M.I./A.A.B.* **164**, Sept. (1976)
Citrus tristeza	140 ± 10		Bar-Joseph, *et al*, *Phytopatholgy* **60**, 75 (1970)
Clover yellow mosaic	125	3.1	Bos, L., *C.M.I./A.A.B.* **111**, July (1973)
Cocksfoot mild mosaic	105 ± 1		Huth & Paul, *C.M.I./A.A.B.* **107**, Oct. (1972)
Cocksfoot mottle	118		Catherall, *C.M.I./A.A.B.* **23**, Oct. (1970)
Cowpea chlorotic	88.3	5.87	Bancroft, J.B., *C.M.I./A.A.B.* **49**, June (1971)

Table A-5 Sedimentation rates and ultraviolet absorbances of some representative viruses [a] (Continued)

Virus	S^{W}_{20}	Absorbance [1mg/mL] – 1 cm at 260 nm	Reference
Cowpea mosaic	58, 95, 115	6.2, 10.0, 8.1	Van Kammen, *C.M.I./A.A.B.* **47**, June (1971)
Cucumber mosaic	98	5.0	Gibbs, A.J. & Harrison, B.D., *C.M.I./A.A.B.* **1**, June (1970)
Cucumber necrosis	113		Dias & Doanne, *Can. J. Bot.* **46**, 47 (1968)
Dahlia mosaic	254		Brunt, A.A., *C.M.I./A.A.B.* **51**, June (1971)
Echtes Ackerbohnenmosaik	98, 119	7.7	Gibbs, A.J. & Paul, H.L., *C.M.I./A.A.B.* **20**, June (1970)
Foot and mouth disease	140	7.6	Bachrach, H.L., *Virology* **25**, 532 (1965)
Grapevine chrome mosaic	92, 117		Marletti, C.P., *C.M.I./A.A.B.* **103**, Oct. (1972)
Henbane mosaic	160		Govier, D.D., *C.M.I./A.A.B.* **95**, June (1971)
Influenza	700		Friedewald & Pickels, *J. Exptl. Med.* **79**, 301 (1944)
Lettuce necrotic yellow	950		Harrison & Crowley, *Virology* **26**, 290 (1965)
Lily symptomless	172		Allen, T.C., *C.M.I./A.A.B.* **96**, Oct. (1972)
Maize dwarf mosaic	155		Bancroft, *et al*, *Phytopathology*, **56**, 474 (1966)
Maize dwarf mosaic B	171 ± 3	2.7	Brakke & Langenberg, unpublished data
Maize rough dwarf	400		Lovisolo, O., *C.M.I./A.A.B.* **73**, Oct. (1971)
Narcissus mosaic	114		Mowat, W.P., *C.M.I./A.A.B.* **45**, June (1971)
Okra mosaic	T 42, B 106	(T&B) 9	Givord, L. & Koenig, R., *C.M.I./A.A.B.* **128**, July (1974)
Papaya mosaic	118.7	2.85	Hiebert, *Phytopathology* **60**, 1295 (1970)
Parsnip mosaic	149		Murant, A.F., *C.M.I./A.A.B.* **91**, June (1972)
Peanut mottle		2.6	Bock, K.R. & Kuhn, C.W., *C.M.I./A.A.B.* **141**, Oct. (1975)
Peanut stunt	98	4.8	Mink, G.I., *C.M.I./A.A.B.* **92**, June (1972)
Pea enation mosaic	100, 120	7.5	Shepherd, R.M, *C.M.I./A.A.B.* **25**, Oct. (1970)
Pea seed borne mosaic	154	2.5	Hampton, R.O. & Mink, G.I., *C.M.I./A.A.B.* **146**, Oct. (1975)
Pepper veinal mottle	155		Brunt & Kenter, *C.M.I./A.A.B.* **104**, June (1972)
Phalia-cauliflower mosaic	216		Brunt, *Virology* **28**, 778 (1966)
Polio	160		Schaffer & Schwerdt, *Adv. In Virus Res.* **6**, 159 (1959)
Popular mosaic	165		Biddle, *C.M.I./A.A.B.* **75**, Oct. (1971)
Potato aucuba mosaic	130	2.6	Kassanis, B. & Govier, D.A., *C.M.I./A.A.B.* **98**, Oct. (1972)
Potato virus X	118	2.97	Various
Potato virus Y	154		Delgado & Grogan, *Phytopathology* **56**, 1397 (1966)

Table A-5 Sedimentation rates and ultraviolet absorbances of some representative viruses [a] (Continued)

Virus	S^{W}_{20}	Absorbance [1mg/mL] – 1 cm at 260 nm	Reference
Potato Yellow Dwarf	900		Brakke, M.K., *Virology* **6**, 96, (1958)
Proteins		0.6-1.5	Various
Prunus necrotic ringspot	79-97, 107-119		Fulton, R.W., *C.M.I./A.A.B.* **121**, July (1973)
Radish mosaic	T 57, M 97, B 116		Campbell, R.N., *C.M.I./A.A.B.* **121**, July (1973)
Red clover mottle	60, 101,127	13.0	Gibbs, *et al*, *Ann. Appl. Biol.* **61**, 99 (1968)
Red clover vein mosaic	160		Varma, Anupam, *C.M.I./A.A.B.* **22**, Oct. (1970)
Ribonucleic acid		22-25	Various
Rice dwarf	510		Lider, *et al*, *C.M.I./A.A.B.* **102**, Oct. (1972)
Rice tungro	175		Galvez, G.E., *C.M.I./A.A.B.* **67**, Oct. (1971)
Rice yellow mottle	109	6.5	Bakker, U., *C.M.I./A.A.B.* **149**, Oct. (1975)
Rod-shaped viruses		2.5-3.0	Various
Saguaro cactus virus	118	6.0	Nelson, M.R. & Tremaine, J.R., *C.M.I./A.A.B.* **148**, Oct. (1975)
Satellite virus	50, 169, 231, 332	6.5	Kassanis, *C.M.I./A.A.B.* **15**, June (1970)
Scrophularie	T 54, B 116	(T & B) 8	Bercks, R., *C.M.I./A.A.B.* **113**, July (1973)
Shope papilloma	280		Schachmann, H.K., *J. Am. Chem. Soc.* **73**, 4453
Simian virus 40	240		Black, *et al*, *Virology* **24**, 381 (1964)
Soil-borne wheat mosaic	172, 211	3.1	Brakke, M., *C.M.I./A.A.B.* **77**, Oct. (1971)
Southern bean mosaic	115	5.8	Various
Sowbane mosaic	104 ± 2	4.9	Kodo, C.I., *C.M.I./A.A.B.* **64**, Oct. (1971)
Spherical virus		4.8-13	Various
Squash mosaic	T 57, M 95, B 118		Campbell, R.N., *C.M.I./A.A.B.* **43**, June (1971)
Sugarcane mosaic	175 ± 55, 168 ± 65, 155 ± 3, 148 ± 25		Various
Sunn hemp mosaic	20–50, 70–80 187	3.2	Kassanis, B. & Varma, A., *C.M.I./A.A.B.* **153**, Oct. (1975)
T_2, T_4, T_6 Phages	700–900, 1000		Cummings, D.J., *Virology* **23**, 408 (1964); Hook, A.I., *et al*, *J. Biol. Chem.* **165**, 241 (1966)
T_3-B Phage	470		Cummings, D.J., *Virology* **23**, 408 (1964)
T_3-C Phage	366		Cummings, D.J., *Virology* **23**, 408 (1964)
T-5 Phage	469–606		Cummings, D.J., *Virology* **23**, 408 (1964)
T-7 Phage	470		Cummings, D.J., *Virology* **23**, 408 (1964)
Tobacco etch	154	2.41	Purcifull, *Virology* **29**, 8 (1964)

Table A-5 Sedimentation rates and ultraviolet absorbances of some representative viruses [a] (Continued)

Virus	S^{W}_{20}	Absorbance [1mg/mL] – 1 cm at 260 nm	Reference
Tobacco mosaic	194	3.0	Zaitlin, M. & Israel, H., *C.M.I./A.A.B.* **151**, Oct. (1975)
Tobacco necrosis	118	5.0–5.5	Kassanis, *C.M.I./A.A.B.* **151**, June (1970)
Tobacco necrosis satellite	50	6.5	Kassanis, B., *C.M.I./A.A.B.* **15**, June (1970)
Tobacco rattle	Short 155–243 Long 300	3.0	Harrison, B., *C.M.I./A.A.B.* **12**, June (1970)
Tobacco ringspot	T 53, M 91, B 126, B 10.0		Stace-Smith, R., *C.M.I./A.A.B.* **17**, June (1970)
Tobacco streak	90–113	5.1	Fulton, R.W., *C.M.I./A.A.B.* **44**, June (1971)
Tomato aspermy	98–100		Hollings & Stone, *C.M.I./A.A.B.* **78**, Oct. (1971)
Tomato bushy stunt	131–140	4.5	Martelli, *C.M.I./A.A.B.* **69**, Oct. (1971)
Tomato ringspot	T 53, B 126–128	B 10.0	Stace-Smith, R., *C.M.I./A.A.B.* **18**, June (1970)
Tomato spotted wilt	530, 583		Best, *Adv. Virus Res.* **13**, 68 (1968) Black, Brakke & Vatten, *Virology* **20**, 120 (1963)
Tomato blackring	97		Harrison & Nixon, *Virology* **12**, 104-117 (1960)
Turnip crinkle	129		Hollings & Stone, *C.M.I./A.A.B.* **109**, Oct. (1972)
Turnip yellow mosaic	T 53–54, B 116–117	T 0.96, B 9.6	*C.M.I./A.A.B.* **2**, June (1970)
Wheat streak mosaic	165		Brakke, M.K., *Virology* **6**, 96 (1958)
Wheat streak mosaic (American)	900		Yamazaki & Kaesberg, *Biochem. Biophys. Acta* **51**, 9 (1961)
Wheat striate mosaic	900	3.1	Sinha, R.C. & Behki, R.M., *C.M.I./A.A.B.* **99**, Oct. (1972)
White clover mosaic	119	3.6	Berks, R., *C.M.I./A.A.B.* **41**, June (1971)
Wild cucumber mosaic	53, 119	3.6	Fry, *et al*, *Phytopathology* **50**, 175 (1960)
Wound tumor	510	1.0	Black, L.M., *C.M.I./A.A.B.* **34**, Oct. (1970)

a. Many viruses are multicomponent, that is they have more than one virion-like particles. These particles may have different amounts of nucleic acid or no nucleic acid. The particles are commonly referred to the sedimentation rates are usually given for the individual particles. With rod-shaped viruses, multicomponent particles are referred to as "short" and "long."