Model 175
Tube Cell

Instruction Manual

Catalog Number
165-1980

For Technical Service
Call Your Local Bio-Rad Office or
in the U.S. Call **1-800-4BIORAD**
(1-800-424-6723)

BIO-RAD

Note

To insure best performance from the Model 175 Tube Cell, become fully acquainted with these operating instructions before using the cell to separate samples. Then assemble and disassemble the cell completely without casting a gel. After these preliminary steps, you should be ready to cast and run a gel.

Bio-Rad also recommends that all Model 175 Tube Cell components and accessories be cleaned with a suitable laboratory cleaner (such as Bio-Rad Cleaning Concentrate, catalog number 161-0722) and rinsed thoroughly with distilled water, before use.

Model _____

Catalog No. _____

Date of Delivery _____

Warranty Period _____

Serial No. _____

Invoice No. _____

Purchase Order No. _____

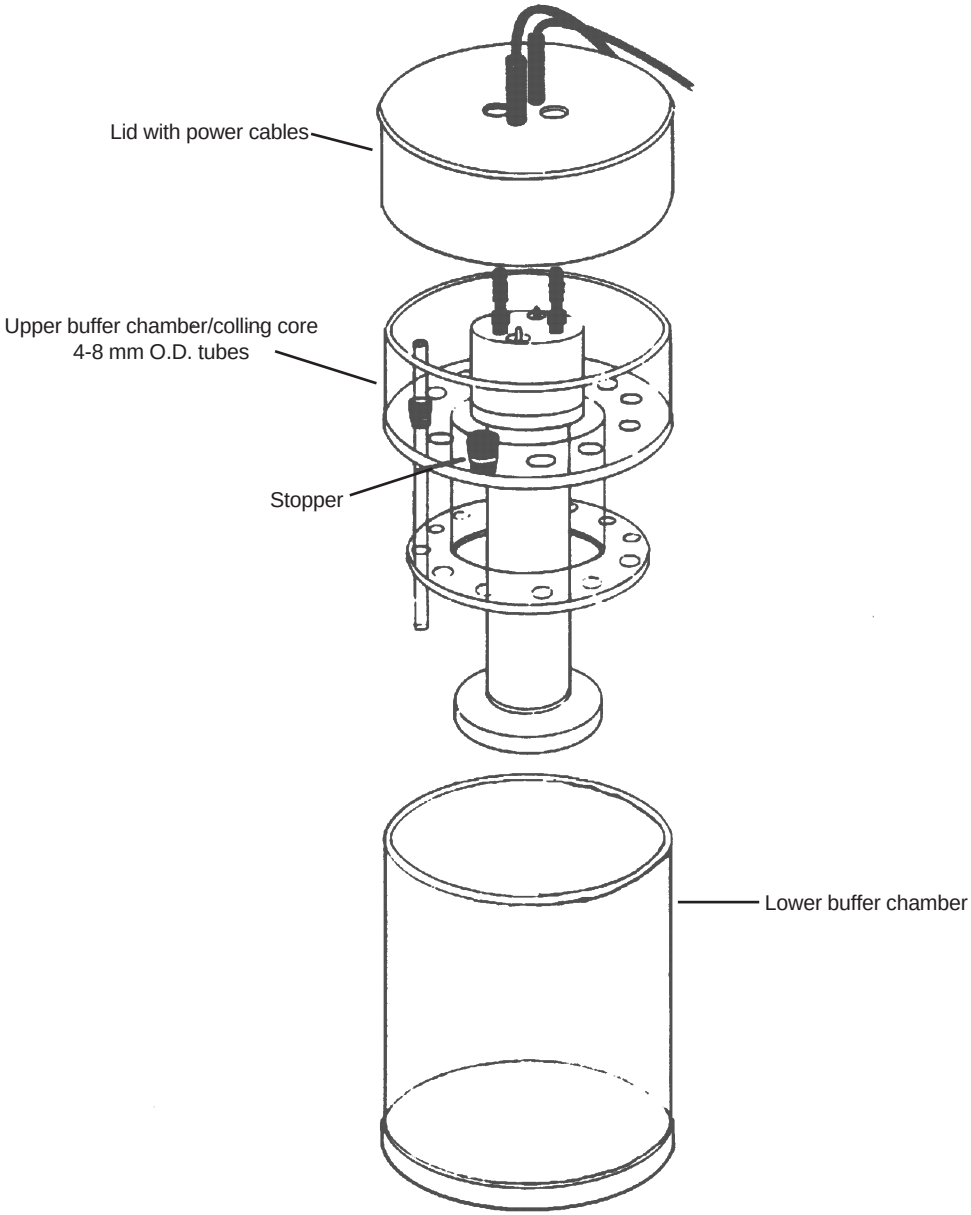
Warranty

Bio-Rad Laboratories warrants the Model 175 Tube Cell against defects in materials and workmanship for 1 year. If any defects occur in the instrument during this warranty period, Bio-Rad Laboratories will repair or replace the defective parts free. The following defects, however, are specifically excluded:

1. Defects caused by improper operation.
2. Repair or modification done by anyone other than Bio-Rad Laboratories or an authorized agent.
3. Use of fittings or other spare parts supplied by anyone other than Bio-Rad Laboratories.
4. Damage caused by accident or misuse.
5. Damage caused by disaster.
6. Corrosion due to use of improper solvent or sample.

For any inquiry or request for repair service, contact Bio-Rad Laboratories after confirming the model and serial number of your instrument. **Note:** Clean all components of the tube cell with suitable laboratory cleaner (such as Bio-Rad's Cleaning Concentrate, catalog number 161-0722), and rinse thoroughly with distilled water, before use.

Table of Contents



Model 175 Tube Cell Components

Section 1
Introduction

1.1 General Information

Bio-Rad's Model 175 Tube Cell can be used for all common tube gel electrophoretic techniques, including discontinuous buffer systems such as Laemmli SDS electrophoresis, non-denaturing "native" buffer systems, preparative tube gel applications, and tube gel isoelectric focusing as in the first dimension of 2-D electrophoresis.

The combination cooling buffer/upper core chamber of the Model 175 Tube Cell accommodates twelve 4-8 mm OD glass tubes (1-6 mm diameter gels) and is useful for most analytical and electrofocusing applications.

The cooling core allows tube gels to be run below ambient temperature. This can be useful in many non-denaturing or preparative systems where recovery of active molecules is necessary. The core can be connected to any circulating water chiller and will maintain the lower buffer and gels at a reset temperature. The cooling core also houses the upper and lower electrodes which have been recessed to prevent breakage.

The lid of the Model 175 Tube Cell fully encloses the upper buffer chamber and overlaps the lower chamber so that the upper buffer chamber/cooling core cannot be removed without first removing the lid. Removing the lid disconnects the unit from the power source, preventing accidental electrical mishap. The lid can only be placed on the Model 175 Tube Cell in "On" orientation, which prevents unintentional reversal of the anode/cathode connections.

1.2 Specifications

Construction:	
Upper buffer chamber/cooling core, lower buffer chamber, lid	Fabricated acrylic
Electrical leads	flexible, coiled with banana plugs
Electrodes	Platinum wire 0.010 inch diameter (0.254 mm)
Shipping weight	2.7 kg (5.9 lb)
Overall size	7-5/8 inch diameter x 11.0 inch length
Cooling core maximum flow rate	2 liters/min
Power Limit	12 Watts
Voltage Limit	3,000 VDC

Note: Cell components are not compatible with chlorinated hydrocarbons (e.g., chloroform), aromatic hydrocarbons (e.g., toluene, benzene), acetone. Use of such organic solvents voids all warranties. Call 1-800-4BIORAD for technical information regarding chemical compatibility of Model 175 Tube Cell components with various laboratory reagents.

1.3 Safety

Power to the Model 175 Tube Cell is to be supplied by an external DC voltage power supply. **This power supply must be ground isolated in such a way that the DC voltage output floats with respect to ground.** All of Bio-Rad's power supplies meet this important safety requirement. Regardless of which power supply is used, the maximum specified operating parameters for the cell are:

3000 VDC ⎓	maximum voltage limit
12 Watts	maximum power limit
50°C	maximum ambient temperature limit

Current to the cell, provided from the external power supply, enters the unit through the lid assembly, providing a safety interlock to the user. Current to the cell is broken when the lid is removed. **Do not attempt to circumvent this safety interlock, and always turn the power supply off before removing the lid, or when working with the cell in any way.**

Important

This Bio-Rad instrument is designed and certified to meet IEC1010-1* safety standards. Certified products are safe to use when operated in accordance with the instruction manual. This instrument should not be modified in any way. Alteration of this instrument will:

- Void the manufacturer's warranty
- Void the IEC1010-1 safety certification
- Create a potential safety hazard

Bio-Rad is not responsible for any injury or damage caused by the use of this instrument for purposes other than those for which it is intended or by modifications of the instrument not performed by Bio-Rad or an authorized agent.

*IEC1010-1 is an internationally accepted electrical safety standard for laboratory instruments.

Section 2
General Operating Instructions

Note: Since several applications can be performed using the Model 175 Tube Cell, discussion in this section is a general description of the operation of the unit which can be applied to most buffer systems. Formulations for SDS-PAGE (Laemmli) gels and isoelectric focusing (IEF) gels are included in Section 4 of this manual.

2.1 Preparation of Gels

1. Prepare the monomer solution by combining all reagents except the initiators (usually ammonium persulfate and TEMED). Degas under vacuum for at least 15 minutes.
2. Mark the tubes at the level to which the acrylamide should be poured.
3. Wrap a double thickness of Parafilm® laboratory film over the opposite end of each gel tube.
4. Place each glass tube in a leveled tube gel preparation rack (such as Bio-Rad's Model 225 Tube Gel Casting Stand, catalog number 165-2020), with the parafilm end down.

5. Add initiators to the monomer solution, mix by swirling, and pour the tube gels.

    a. For capillary tube gels ≤ 2.5 mm I. D., gels must be poured with a syringe and a fine gauge needle long enough to reach the bottom of the tubes (catalog number 165-1943). To prevent bubbles at the bottom of the capillary gels, apply a little extra force on the syringe initially to remove trapped air.

    b. For tube gels ≥ 2.5 mm I. D., gels may be poured as above, or with a long Pasteur pipet and bulb.

6. Overlay each gel with a suitable overlay solution (see Section 4). Allow to polymerize approximately 1 hour.

2.2 Tube Placement

1. After the gels have polymerized, remove the Parafilm and rinse the top and bottom of each gel with distilled water.
2. Push each cast tube gel into an appropriate size grommet, so that the application surface of the gel is visible above the grommet.
3. Place each tube and grommet assembly into a hole in the upper buffer chamber from the top. Firmly seat the stopper to provide a good seal.
4. Fill any unused holes with a solid stopper.

2.3 Lower Buffer Reservoir Preparation

1. Place a magnetic stir bar into the bottom of the lower buffer chamber.
2. Fill the lower reservoir with the lower reservoir buffer. With the upper buffer chamber/cooling core in place, the volume required to fill the reservoir almost to the top is approximately 3.5 liters. To provide maximum cooling conditions, the lower buffer should cover the entire separating gel.
3. Lower the upper chamber/cooling core into the lower chamber and adjust the lower buffer volume as needed.
4. Add approximately 600 ml of upper buffer to the upper buffer chamber.
5. Place the entire Model 175 Tube Cell on a magnetic stirrer.

2.4 Sample Preparation and Application

Note: During sample preparation and loading the lower buffer may be chilled to a preset temperature by connecting a circulating cooler to the ports on the central cooling core.

1. Prepare the sample in an appropriate sample buffer. In general, the sample buffer should contain 5-10% sucrose or glycerol to make the sample denser than the upper buffer. In most buffer systems, a tracking dye is added to the sample buffer to indicate when electrophoretic run is complete.
2. Carefully apply each sample to the top of the gel using a syringe and needle, or, for large diameter gels (≥ 3 mm I.D.), using an automatic pipettor. The sample should be applied as close to the gel surface as possible, and in a careful manner so that no air bubbles are introduced.

2.5 Sample Electrophoresis

1. Place the lid on the Model 175 Tube Cell and connect the electrical leads to a suitable DC power supply such as Bio-Rad's PowerPac 3000 or PowerPac 1000 power supply.
2. If cooling is desired, connect the cooling core ports to a circulating cooler or to a tap water line. The core may also be filled with any common anti-freeze or methanol: water (20:80) to act as a heat sink.
3 Turn on the power supply and set the power conditions recommended for that application.

2.6 Extruding Tube Gels

1. After electrophoresis, shut off the power supply, disconnect the leads, and remove the lid of the tube cell.
2. Lift out the upper chamber/cooling core and pour off the upper buffer. Remove the gel tubes from the upper chamber and place them in the tube gel casting stand to prevent mix-up.
3. Attach a long, fine, beveled needle (catalog number 165-1944) to a 1 or 3 ml plastic syringe filled with distilled water. Rim the upper and lower few millimeters of each gel by inserting the needle between the gel and the glass tube (point against the glass wall) while forcing distilled water through the syringe and needle. Turn the gel tube so that the entire circumference is rimmed.
4. Attach a piece of tubing to a 1 or 3 ml syringe and to the outside wall of the glass tube on the top end of the tube gel to be extruded. Using the syringe filled with distilled water, apply a firm, even pressure to start the gel extruding from the tubes. As the gel moves further out of the tube, apply less pressure so that the rate of extrusion remains constant. Do not extrude to quickly. Only slight pressure is required to remove the last 1-2 cm of gel from the tube.
5. Extrude the gels into plastic or glass storage tubes or into small beakers for equilibration, fixation, etc. Bio-Rad's Incubation Tray (catalog number 170-4037) makes a convenient container for the gels.

Section 3
Maintenance of Equipment

3.1 Model 175 Cell Chamber, Stoppers, Grommets

Rinse with distilled water after every use. Clean with a laboratory detergent (catalog number 161-0722), and rinse with dH_2O.

3.2 Glass Tubes

After use, rinse with laboratory detergent solution, scrub out if possible, then rinse with distilled H_2O. For a more stringent cleaning, soak the tubes in a chromic/sulfuric acid solution overnight and then rinse with distilled water. Dry in a forced air or vacuum oven before use.

Warning: Exercise extreme caution for acid cleaning: wear safety glasses, a lab coat, and rubber gloves. Keep a container of $NaCO_3$ nearby to neutralize spills.

Section 4
Protocols

4.1 Reagents and Gel Preparation of SDS-PAGE Slab Gels (Laemmli buffer system*)

Stock Solutions

A. Acrylamide/bis (30% T, 2.67% C)

146 g acrylamide (29.2 g/100 ml)

4 g N' N'-bis-methylene-acrylamide (0.8 g/100 ml)

Make to 500 ml with distilled water. Filter and store at 4 °C in the dark (30 days maximum).

Or substitute Bio-Rad's preweighed acrylamide/bis.

37.5:1 mixture (catalog number 161-0112, 30 g)

(catalog number 161-0106, 200 g)

150 g acrylamide/bis 930 g/100 ml) to 500 ml with dH_2O.

B. 1.5 M Tris-HC1, pH 8.8

54.45 g Tris base (18.15 g/100 ml)

~150 ml distilled water

Adjust to pH 8.8 with 1 N HC1. Make to 300 ml with distilled water and store at 4 °C.

C. 0.5 M Tris-HC1, pH 6.8

6 g Tris base

~60 ml distilled water

Adjust to pH 6.8 with 5-10 N HC1. Make to 100 ml with distilled water and store at 4° C.

D. 10% SDS

Dissolve 10 g SDS in water with gentle stirring and bring to 100 ml with dH_2O.

*Laemmli, U. K., Nature, 227, 680 (1970)

E. Sample buffer (store at room temperature)

Distilled water	4.0 ml
0.5 M Tris-HC1, pH 6.8	1.0 ml
Glycerol	0.80 ml
10% (w/v) SDS	1.6 ml
2-mercaptoethanol 0.4 ml	0.4 ml
0.05% (w/v) bromophenol blue 0.2 ml	
	8.0 ml

Dilute the sample at least 1:4 with sample buffer, and heat at 95 °C for 4 minutes.

F. 5x electrode (running) buffer, pH 8.3 (enough for 10 runs)

Tris base	45 g	(15 g/l)
Glycine	216 g	(72 g/l)
SDS	15 g	(5 g/l)
		to 3 liters with dH2O

Store at 4 °C. Warm to 37 °C before use if precipitation occurs.

Dilute stock solution 1:5 with distilled water for electrophoresis run.

4.2 Separating Gel Preparation--0.375 M Tris, pH 8.8

	12%[a]	7.5%[b]
Distilled water	33.5 ml	48.5 ml
1.5 M Tris-HCI, pH 8.8	25.0 ml	25 ml
10% (w/v) SDS stock	1.0 ml	1.0 ml
(store at room temperature)		
Acrylamide/bis (30% stock)	40.0 m	25.0 ml
*10% ammonium persulfate	500 µl	500 µl (0.05%)
TEMED	50 µl	50 µl (0.05%)
TOTAL MONOMER+	100 ml	100 ml

* To make the 10% ammonium persulfate solution dissolve 100 mg APS in 1 ml dH_2O.

+ One can prepare any desired volume of monomer solution by using multiples or fractions of the 100 ml recipes.

[a] For SDS treated proteins in the approximate molecular weight range between 10-100 K daltons. Use Bio-Rad's Low MW Standards (catalog number 161-0304) for 12% separating gel.

[b] For SDS treated proteins in the approximate molecular weight range between 10-100 K daltons. Use in conjunction with Bio-Rad's High MW SDS-PAGE Standards (catalog number 161-0303).

To calculate the amount of monomer needed, use the following formula:

$$\left[\frac{(\text{tube diameter in cm})^2}{2} \times 3.14 \times \text{height in cm needed to fill}\right] \times \text{number of tubes}$$

For example, to cast ten 1.5 mm diameter tubes to a height of 14 cm, calculate as follows:

$$\left[\frac{(.15)^2}{2} \times 3.14 \times 14 \text{ cm}\right] \times 10 = 4.9 \text{ ml}$$

It is advisable to add an additional 2-5 ml to the calculated volume.

4.3 Stacking Gel Preparation--4.0% gel, 0.125 M Tris, pH 6.8

Distilled water	6.1 ml	12.2 ml
0.5 M Tris-HCl, pH 6.8	2.5 ml	5 ml
10% (w/w) SDS	100 µl	200 µl
Acrylamide/bis (30% stock)	1.3 ml	2.6 ml
(Degas for 15 minutes at room temperature)		
10% ammonium persulfate (fresh daily)	50 µl	100 µl (0.05%)
TEMED	10 µl	20 µl (0.1%)
TOTAL STOCK MONOMER	10 ml	20 ml

1. To prepare the monomer solutions, combine all reagents, except the APS and TEMED, and deaerate under vacuum for ≥ 15 minutes. To initiate polymerization add the APS and TEMED, and swirl gently to mix.
2. Follow the instructions in Section 2.1-2.4 for setup and casting of the gels.

4.4 Running Conditions*

We recommend that the gels be run under constant current conditions with an appropriate power supply, such as Bio-Rad's PowerPac 3000 or Model 1000/500 power supply. (See Section 5.6 for ordering information.)

Run time is 4 to 5 hours, depending on the length of the gel.

Tube Gel Diameter (i. D.) mm	Current Setting m/A gel
2 mm	0.4 mA/gel
2.5 mm	0.6mA/gel
3 mm	0.9 mA/gel
5 mm	2.5 mA/gel
6 mm	3.6 mA/gel

* Recommended power conditions for optimal resolution with minimal distortion. Conditions can be varied by user.

Note: 1.0 and 1.5 mm tube gels are used primarily for capillary isoelectric focusing.

4.5 2-D Stock Solutions

Note: The pH gradient in these protocols is expanded in the pH 5-7 range. The gradient may be expanded in any range by substituting a Bio-Lyte® ampholyte, with a different range for the ampholyte, in the protocol.

First Dimension IEF Tube Gels

10% Triton X-100

Triton X-100	10 ml
H_2O	90 ml

Stir in a beaker with 5 g AG® 501-X8 mixed bed ion exchange resin (catalog number 142-6424). Store in brown glass bottle at room temperature.

Overlay Buffer

10% Triton X-100, 2.0 ml

Bio-Lyte ampholyte, 5/7, 0.45 ml

Bio-Lyte ampholyte, 3/10, 0.05 ml

ddH_2O up to 10.0 ml

Store at 4 °C in capped tube.

IEF Sample Concentrate

10% SDS, 0.1 ml (for certain applications. SDS may be omitted)

Bio-Lyte ampholyte, 3/10, 0.02 ml

Bio-Lyte ampholyte, 5/7, 0.18 ml

2-Mercaptoethanol, 0.1 ml

Triton X-100 (undiluted), 0.2 ml

Mix well and store at 4 °C in capped tube.

Be sure to vortex well before each use.

IEF Gel Solution

Urea, 48.6 g

ddH_2O, 28.8 ml

Acrylamide/bis acrylamide (30% stock), 11.8 ml

10% Triton X-100, 20.3 ml

Bio-Lyte ampholyte, 5/7, 0.45 ml

Bio-Lyte ampholyte, 3/10, 0.05 ml

Store in 5.5 ml aliquots at -80 °C

Electrolytes

Catholyte — NaOH 2.4 g

H_2O to 600 ml (0.1 N NaOH)

Anolyte — 85% phosphoric acid, 2.4 ml

H_2O to 3.5 liters (0.06%)

These solutions should be freshly degassed before use.

4.6 Preparation of First Dimension IEF Gels

The recommended tube diameter for this procedure is 1.5 mm. If larger diameter tubes are used, more gel solution will be required, e.g., 2 aliquots of IEF gel solution if 3.0 mm I. D. tubes are used.

Warning: Always wear gloves when performing this procedure to prevent exposure to acrylamide.

Thaw an aliquot (5.5 ml) of IEF gel solution and bring it to room temperature. Degas for 15 minutes under vacuum. To the IEF gel solution add:

TEMED	5.5 µl
Fresh 10% ammonium persulfate	7.25 µl

This will be enough gel solution to prepare eighteen 15 cm x 1.5 mm gels.

To calculate the exact volume necessary to cast tube gels, refer to Section 4.2.

4.7 Running Conditions for Capillary (1.0 and 1.5 mm) IEF Tube Gels

IEF is carried out at 400 volts constant voltage for 12-15 hours, followed by 2 hours at 800 volts constant voltage.

Section 5
Equipment and Accessories

5.1 Cell

Catalog Number	Product Description
165-1980	**Model 175 Tube Cell,** 4-8 mm OD tubes

The cell includes the combination upper buffer chamber/cooling core, lower buffer chamber, cell lid with power cables, a complete set of grommets and stoppers (to fit all compatible sizes of glass tubes), a leveling bubble, and instructions. Glass tubes are not included and must be ordered separately.

5.2 Accessories

Catalog Number	Product Description
165-1984	**Grommets and Stoppers,** 4-5 mm OD tubes (12), 1 set
165-1985	**Grommets and Stoppers,** 6-7 mm OD tubes (12), 1 set
165-1986	**Grommets and Stoppers,** 8 mm OD tubes (12), 1 set
165-1943	**Gel Tube Loading Needle,** 18 cm, 22 gauge, blunt tip, Luer hub (for casting monomer in small diameter tubes)
165-1944	**Tube Gel Extrusion Needle,** 26 gauge, beveled tip, Luer hub (for removing gels from tubes)
165-2020	**Model 225 Tube Gel Casting Stand**

5.3 Glass Tubes

Product Description

Catalog Number	ID mm	OD mm	Length mm	Package Quantity
165-3136	1.0	5.0	180	24
165-3137	1.5	6.0	150	24
165-3138	1.5	6.0	180	24
165-3139	2.0	6.5	180	24
165-3155	2.4	4.0	160	24
165-3150	3.0	5.0	125	24
165-3122	5.0	7.0	125	24

5.4 Electrophoresis Chemicals

Catalog Number	Product Description	Quantity per Package
161-0100	**Acrylamide,** 99.9%	100 g
161-0101	**Acrylamide,** 99.9%	500 g
161-0170	**Acrylamide,** 99.9%	1 kg
161-0103	**Acrylamide,** 99.9%	2 kg
161-0122	**Preweighted Acrylamide/Bis,** 37.5:1 mixture	30 g
161-0125	**Preweighted Acrylamide/Bis,** 37.5:1 mixture	150 g
161-0121	**Preweighted Acrylamide/Bis,** 29:1 mixture	30 g
161-0124	**Preweighted Acrylamide/Bis,** 29:1 mixture	150 g
161-0200	**Bis** (N, N'-Methylene-bis acrylamide)	5 g
161-0201	**Bis** (N, N'-Methylene-bis acrylamide)	50 g
161-0716	**Tris**	500 g
161-0719	**Tris**	1 kg
161-0717	**Glycine**	250 g
161-0718	**Glycine**	1 kg
161-0300	**SDS** (sodium dodecylsulfate)	25 g
161-0301	**SDS** (sodium dodecylsulfate)	100 g
161-0302	**SDS** (sodium dodecylsulfate)	1 kg
161-0700	**Ammonium Persulfate**	10 g
161-0610	**Dithiothreitol**	1 g
161-0611	**Dithiothreitol**	5 g
161-0710	**2-mercaptoethanol**	25 ml
161-0800	**TEMED**	5 ml
161-0801	**TEMED**	50 ml
162-0100	**Agarose,** Standard Low -m_r	100 g
162-0102	**Agarose,** Standard Low -m_r	500 g
161-0304	**SDS-PAGE Standards**, 10,000-100,000 MW	

Catalog Number	Product Description	Quantity per Package
161-0303	**SDS-PAGE Standards,** 40,000-250,000 MW	
161-0310	**IEF Standards**	
161-0443	**Silver Stain Kit,** includes 1 bottle oxidizer concentrate, 1 bottle silver reagent concentrate, and 4 bottles developer. Enough to stain approximately 48 (8 x 7 cm) gels.	
161-0400	**Coomassie Blue R-250**	10 g
161-0404	**Bromophenol Blue**	10 g
161-0407	**Triton X-100**	500 ml
161-0730	**Urea**	250 g
161-0731	**Urea**	1 g
161-0722	**Cleaning Concentrate** (50x)	1 kg

Bio-Lyte® Ampholytes

163-1112	**Bio-Lyte Ampholytes,** 3/10, 40%	10 ml
163-1132	**Bio-Lyte Ampholytes,** 3/5, 20%	10 ml
163-1142	**Bio-Lyte Ampholytes,** 4/6, 40%	10 ml
163-1152	**Bio-Lyte Ampholytes,** 5/7 40%	10 ml
163-1162	**Bio-Lyte Ampholytes,** 6/8, 40%	10 ml
163-1172	**Bio-Lyte Ampholytes,** 7/9, 40%	10 ml
163-1182	**Bio-Lyte Ampholytes,** 8/10, 20%	10 ml
142-6424	**AG®501-X8 Mixed Bed Ion Exchange Resin**	500 g

5.5 Power Supplies

165-5056	**PowerPac 3000 Power Supply,** 100/120 VAC
165-5057	**PowerPac 3000 Power Supply,** 220/240 VAC
165-4710	**Model 1000/500 Power Supply,** 110/120 VAC
165-4711	**Model 1000/500 Power Supply,** 220/240 VAC

Section 6
References

6.1 General References

1. Gordon, A. H., *"Laboratory Techniques in Biochemistry and Molecular Biology,"* Vol. 1, Part 1,Work, T.S. and Work, E., eds. North Holland Publishing Co., Amsterdam-London (1975).
2. Maurer, H. R., *"Disk Electrophoresis and Related Techniques of Polyacrylamide Gel Electrophoresis,"* Walter de Gruyter, Berlin-New York (1971).

6.2 Native Gel Systems References

1. Ritchie, R. F., Harter, J. G. and Bayles, T. B., *J. Lab. Clin. Med.,* **68,** 842 (1966).
2. Margolis, J. and Kendrick, K. G., *Anal Biochem.,* **25,** 347 (1968).
3. Ornstein, L. and Davis, B. J., *Ann. N. Y. Acad. Sci.,* **121,** 321 (1964).
4. Reisfeld, R. A., Lewis, U. J. and William, D. E., *Nature,* **195**, 281 (1962).
5. Blattler, D.P., Garner, F., Van Slkye, K. and Bradley, A., *J. Chromatog.,* **64,** 147 (1972).
6. Jeppesen, P. G. N., *Anal. Biochem.,* **58,** 195 (1974).

6.3 SDS Gel System References

1. Shapiro, A. L., Vinuela, E. and Maizel, J. V., *Biochem. Biophys. Res. Commun.,* **28,** 815 (1967).
2. Laemmli, *U. K., Nature,* **227,** 680 (1970).
3. Fairbanks, G., Steck, T. L. and Wallace, D. F. H., *Biochemistry,* **10,** 2606 (1971).
4. Weber, K. and Osborn, M., *J. Biol. Chem.,* **224,** 4406 (1969).
5. Yamada, K. M. and Weston, J. A., *Proc. Nat. Acad. Sci. U.S.A.,* **71,** 3492 (1974).
6. Neville, D. M., *J. Biol. Chem.,* **246,** 6328 (1971).
7. O'Farrell, P. Z., Gold, L. M. and Hauang, W. M., *J. Biol. Chem.,* **248,** 5499 (1973).
8. Studier, F. W., *J. Mol. Biol.,* **79,** 237 (1973).
9. Ferro-Luzzi Ames, G., *J. Biol. Chem.,* **249,** 634 (1974).

6.4 Urea Gel Systems References

1. Kaltschmidt, E. and Wittmann, H. G., *Anal. Biochem.,* **36,** 401 (1970).
2. Swank, R. W. and Muckers, K. D., *Anal. Biochem.,* **39,** 462 (1971).
3. Jakes, K., Zinder, N. D. and Boon, T., *J. Biol. Chem.,* **249,** 438 (1974).
4. Mets, L. J. and Bogorad, L., *Anal. Biochem.,* **57,** 200 (1974).
5. Sherton, C. C. and Wool, I. G., *J. Biol. Chem.,* **249,** 2258 (1974).

6.5 Two-Dimensional IEF/SDS-PAGE Gel Systems References

1. O'Farrell, P. H., *J. Biol. Chem.,* **250,** 4007 (1975).
2. Ferro-Luzzi Ames, G., and Nikaido, K., *Biochem.,* **15,** 616 (1976).
3. Anderson, L. and Anderson, N. G., *Proc. Nat. Acad. Sci U.S.A.,* **74,** 5421 (1977).
4. O'Farrell, P. Z., Goodman, H. M. and O'Farrell, P. H., *Cell,* **12,** 1133 (1977).
5. Garrels, J. I., *J. Biol. Chem.,* **254,** 7961 (1979).
6. Dunn, M. J. and Burghes, A. H. M., *Electrophoresis,* **4:** 97-116 and **4:** 173-189 (1983).

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M1651980 Rev B