Model 385 and 395 Gradient Former

Instruction Manual

Catalog Numbers
165-2000 and 165-2001

BIO-RAD

Table of Contents

Section 1
Introduction

The Model 385 and Model 395 Gradient Formers are primarily used to construct reproducible linear and exponential polyacrylamide gradient gels. Both gradient formers can also be used to construct sucrose density gradients for ultracentrifugation or elution gradients for column chromatography. To obtain optimal chromatography elution gradients, tubing with an inside diameter □ 1/16" must be used.

The Model 385 Gradient Former has a 30-100 ml capacity, making it ideal for the construction of one or two identical standard (16 x 20 x 0.15 cm) polyacrylamide gradient gels, or up to 10 identical miniature (8 x 7 x 0.15 cm) gradient gels. The Model 395 Gradient Former has a 100-700 ml capacity, making it ideal for the construction of up to 12 identical standard (16 x 20 x 0.15 cm) gradient gels using a gel casting chamber.

The mixing and reservoir chambers of both gradient formers have identical dimensions. The outlet from the mixing chamber leads through a peristaltic pump (not required for linear gradients) to a single gel, to a Y for two gels, into a gel casting chamber for multiple gels. The gradient may be cast from the top or bottom of the gel sandwich. When the gradient is formed in a gel casting chamber, the gradient can be cast only from the bottom. Exponential pistons, available as accessories for both gradient formers, are used to fix the volume in mixing chamber to produce concave or convex exponential gradients.

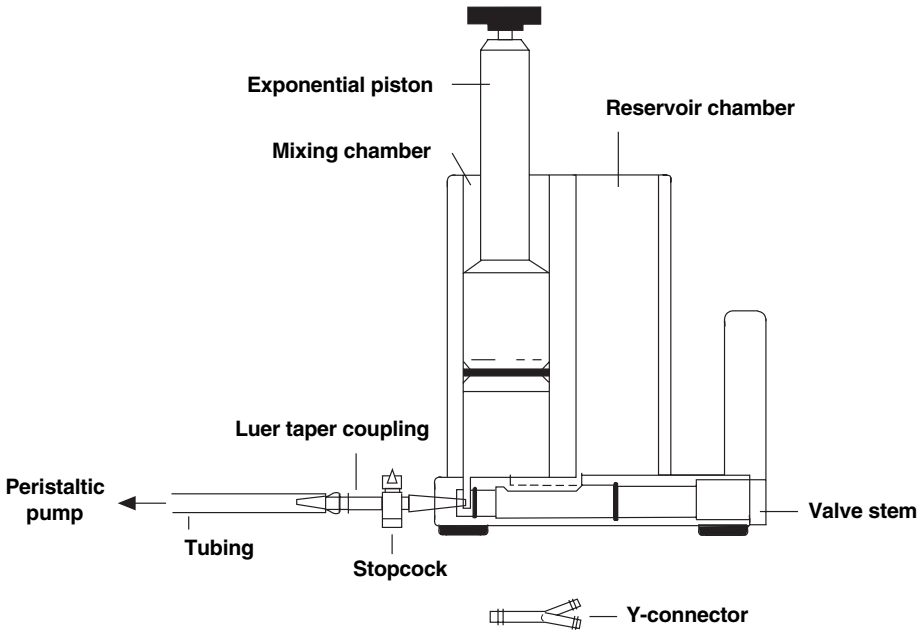

Fig. 1.1. Model 385 and 395 Gradient Formers, major parts.

1.1 Specifications

Model 385 Gradient Former

Overall size	10 cm x 15 cm x 15 cm (with piston in chamber)
Weight	405 g without piston; piston 65 g
Capacity	2 x 56 ml

Model 395 Gradient Former

Overall size	16.5 cm x 11.2 cm x 18 cm (without piston)
Weight	730 g without piston; piston 310 g
Chamber volume	2 x 400 ml
Practical capacity	2 x 350 ml

Construction of Model 385 and 395 Gradient Formers

Body	machined from an acrylic block
Valve stem	Delrin®
Stopcock	one-way plastic stopcock, Luer taper
Piston	Delrin
Tubing	Tygon® 1/8" ID

Section 2
Pouring a Linear Gradient Gel

2.1 Calculating the Chamber Volumes

Each chamber's volume is one-half the total volume of the gel. The volume of the tubing and the stir bar, however, can affect the measurement of the total volume. Section 2.2, step 10, discusses tubing volume.

Adding the stir bar to the mixing chamber increases the volume in the mixing chamber. If not compensated for, this increased volume would divide itself between the two chambers when the valve stem was opened. However, when the stirring motor is turned on, a vortex is created, which compensates for the increase in mixing chamber volume, since the bottom of the vortex becomes the level at which the other chamber equalizes.

To calculate the volume of solution required for each chamber, multiply the spacer thickness in cm by the length of the gel in cm, by the width of the gel in cm, and divide by 2.

2.2 Pouring the Gel from the Top

The heavy solution is poured into the mixing chamber since it enters the sandwich first, flowing to the bottom. The light solution, which flows into the gel last, is poured into the reservoir chamber.

Note: We advise you to familiarize yourself thoroughly with the sequence of operations starting with step 6. The best guarantee of reproducibility from gradient to gradient is careful technique on the part of the operator.

1. Set up the gel sandwich in the usual manner.
2. Arrange the tubing in the peristaltic pump so there is as short a distance as possible between the stopcock opening and the pump, and between the pump and the gel sandwich. Cut the tubing as necessary.
3. Attach the needle to the end of the tubing and tape it to the top of the gel sandwich near the center.

If a peristaltic pump is not used, the level of the gradient former stopcock must be above the top of the gel sandwich by a distance sufficient to create a hydrostatic head large enough to pour the entire volume of the gel within 10 minutes from the time the initiators are added to the light solution. All the acrylamide should be in the gel sandwich before polymerization begins, so that polymerization will be uniform throughout the gel.

Rather then using a needle on the end of the tubing, you may cut the end of the tubing at an angle before taping it to the sandwich. Face the tapered opening toward the glass plate.

4. Place a 1" stir bar (Model 385) or 2" stir bar (Model 395) in the mixing chamber (with valve stem closed).
5. Degas the heavy and light solutions.
6. Add the initiators to the light solution, swirl it 8 to 10 times, and pour it into the reservoir chamber. (This is the start of the 10 minutes.) Leave the valve stem closed.
7. Add the initiators to the heavy solution, swirl it 8 to 10 times, and pour it into the mixing chamber.
8. Start the stirring motor and adjust the speed so that you get good mixing and still the bottom level of the vortex matches the acrylamide level in the reservoir chamber.
9. Quickly open the valve stem and stopcock, and turn on the peristaltic pump.

   Note: The peristaltic pump must be able to pump the whole gradient within 10 minutes from the time you add the initiators to the first solution. This is generally in the range of 5 to 10 ml/min for the Model 385 and 70 to 100 ml/min for the Model 395.
10. Run the pump until all the solution is pushed into the sandwich.

    If a peristaltic pump is not used, the acrylamide will flow down the tubing to the gel sandwich by gravitational force when you open the stopcock.

    Note: If tubing is left dangling below the top of the sandwich, you will have to lift it up and drain it into the top of the gel sandwich to get the entire volume of acrylamide into the sandwich. The tubing volume should not be a problem because the tubing will retain a negligible amount of acrylamide following gradient formation.
11. Overlay the acrylamide with water or water saturated isobutanol. For a continuous buffer system, insert a comb.

    Note: Immediately after the gradient is cast, the system must be flushed with water to prevent polymerization of residual acrylamide within the gradient former or pumping system.
12. Immediately remove the needle (or tubing) from the top of the sandwich and transfer to a waste receptacle. Pour rinse water into both the mixing and reservoir chambers and turn the peristaltic pump on to its maximum speed. The water should immediately flow from the gradient former, through all connectors and tubing of the pumping system, and out the needle (or tubing) to a waste receptacle.

2.3 Pouring the Gel from the Bottom

In this case, the light solution goes in the mixing chamber, and the heavy solution goes in the reservoir. The light solution enters the gel from the bottom and travels to the top, and the heavy solution follows, filling the bottom of the gel.

1. Set up the gel sandwich, gradient former, tubing, and peristaltic pump as described in Section 2.2, steps 1, 2, 4, and 5. For a continuous gradient, insert the comb at an angle.

2. Insert the needle into the sandwich through the gasket on the bottom of the casting stand.
3. To start the linear gradient, use the procedure described in Section 2.2, steps 6-9, except pour the light solution into the mixing chamber and the heavy solution into the reservoir.
4. After the stirring motor is turned on, the vortex level adjusted, the valve stem and stopcock opened, run the peristaltic pump until the last of the acrylamide enters the needle fitting. Do not allow air bubbles to enter the gel, as this could cause the mixing of the gradient. Remove the needle from the bottom of the casting stand.
5. Overlay the gel. For a continuous gel, the comb, which should be positioned at an angel between the gel sandwich, is straightened and inserted fully to form the wells.
6. Rinse the residual acrylamide from the gradient former, tubing, and needle as described in Section 2.2, step. 12.

Section 3
Pouring an Exponential Gradient Gel

In some cases, the number of proteins (polypeptides) in a certain area of the gel will justify pouring an exponential gradient. Whether or not a linear gradient gives adequate resolution is determined empirically for each sample. If there are many bands near the top of the gel, then a concave gradient is indicated, whereas if there are many bands toward the bottom of the gel, a convex gradient should be poured. (See Theory of Linear and Exponential Gradient Gels, Section 7, especially Figures 7.3 and 7.5). The Model 385 gradient former piston (catalog number 165-2006) or the Model 395 gradient former piston (catalog number 165-2005) limits the volume of the mixing chamber so that either a concave or a convex gradient can be formed.

3.1 Concave Exponential Gradient Gels

Concave gradient gels are formed by delivering the acrylamide from the top, with the small volume of heavy solution in the mixing chamber and the large volume of the light solution in the reservoir chamber. See Figure 7.2.

1. Calculate the chamber volumes.

   Calculate the volume of the gel (spacer thickness in cm x gel width in cm x gel length in cm). The volume of the heavy solution, for the mixing chamber, is one-fourth the total volume of the gel. The volume of the light solution, for the reservoir chamber, is equal to the total volume of the gel. Because the volume of the mixing chamber is fixed, not all of the gel solution will be delivered to the gel sandwich. One-fourth the volume will remain in the mixing chamber at the end of the delivery and must be discarded.
2. Set up the equipment as in Section 2.2, steps 1-4.
3. Mix and degas the small volume of heavy acrylamide solution and the large volume of light acrylamide solution, add the initiators to the light acrylamide solution, swirl 8 to 10 times, and pour into the reservoir chamber, keeping the valve stem closed.
4. Add the initiators to the heavy solution, swirl 8 to 10 times, and pour it into the mixing chamber.
5. immediately fix the volume of the mixing chamber by inserting the piston into the chamber to 1 cm above the level of the acrylamide and tightening the screw top handle to hold the piston in place.

6. Quickly turn on the stirring motor (to a speed which gives good mixing without foaming), open the valve stem and the stopcock, and start the peristaltic pump (see Note on the speed of the peristaltic pump).
7. As the pump removes acrylamide from the mixing chamber, the reservoir solution will be drawn into the mixing chamber and the gradient will be formed.
8. When the acrylamide solution reaches the desired level in the gel sandwich, stop the pump, remove the tubing from the gel sandwich, and transfer it to a waste receptacle. Overlay the gel with water or water-saturated isobutanol. For a continuous system, add the comb to form the wells.
9. Remove the exponential piston from the mixing chamber and restart the pump to remove any remaining acrylamide. When the chambers are empty, add rinse water to both the mixing and reservoir chambers and flush out the system as described in Section 2.2, step 12.

3.2 Convex Exponential Gradient Gels

Convex gradient gels are formed by delivering the acrylamide from the bottom, with the small volume of light solution in the mixing chamber and the large volume of heavy solution in the reservoir chamber (see Figure 7.4).

1. Calculate the volumes of light and heavy solutions as in Section 3.1, except that the light solution will be 1/4 the total gel volume and the heavy solution will be equal to the total gel volume.
2. Set up the equipment as described in Section 2.3, with the needle entering the gel sandwich from the bottom through the casting stand gasket. For a continuous gradient, insert the comb into the sandwich at an angle.
3. Mix and degas the small volume of light acrylamide solution and large volume of heavy acrylamide solution.
4. Add the initiators to the light solution, swirl 8 to 10 times, and pour it into the mixing chamber, leaving the valve stem closed.
5. Immediately fix the volume with the piston as described in Section 3.1, step 5.
6. Add the initiators to the heavy solution, swirl 8 to 10 times, and pour into the reservoir.
7. Cast the gradient as described in Section 3,1, steps 6-8.
8. Rinse out the system as described in Section 3.1, step 9.

Section 4
Pouring Two Gradient Gels

Double the volumes of heavy and light solution, and use the Y-connector provided. The procedures are the same as above. It is essential that the tubing from the Y to the two gels be the same length, and that the rate of flow be the same from each needle. We advise that this be checked with water and two measuring cylinders ahead of time.

Note: It is difficult to attain reproducible gradients when pouring two gradient gels.

Section 5
Pouring Multiple Gradient Gels in a Gel Casting Chamber

Note: Gel casting chambers are designed for bottom filling, therefore concave exponential gradient gels can not be found using a gel casting chamber.

1. The total gel volume required depends on several factors, including gel thickness, number of gels, gel length, and amount of space-filling. To make a precise determination of the volume required for a particular application, set up the casting chamber with the glass plates, spacers, and combs (for continuous gradients) to form the required number of gel sandwiches and measure the volume of deionized distilled water required to fill it to the desired level. Then disassemble the chamber and rinse and dry the parts.

2. Calculate the chamber volumes,. For a linear gradient, each chamber's volume is one-half the total gel volume required. For a convex exponential gradient, the light solution in the mixing chamber will be one-fourth the total gel volume and heavy solution in the reservoir chamber will be equal to the total gel volume.

3. Reassemble the multi-gel casting chamber as described in step 2. Be sure the glass plates and spacers are clean and dry.

4. Arrange the tubing in the peristaltic pump so there is as short a distance as possible between the stopcock opening and the pump, and between the pump and the inlet to the gel casting chamber. Attach a 3 cm piece of tubing to the gel casting chamber inlet with a pinch clamp to close it off. Attach one end of a Luer taper coupling to this 3 cm tubing and attach the tubing from the pump to the other end of the Luer taper coupling. Cut the tubing as necessary.

5. For a linear gradient, set up the gradient former as described in Section 2.2, steps 4-9, except pour the light solution into the mixing chamber and the heavy solution into the reservoir chamber. For a convex exponential gradient, set up the gradient former as described in Section 3.2, steps 3-7.

6. Run the pump so that the acrylamide flows through the tubing into the gel casting chamber, until the acrylamide reaches the desired level in the gel sandwiches. Stop before any air enters the gel casting chamber. For bottom filling of linear gradient, the tubing should contain a negligible amount of acrylamide following gradient formation. For bottom filling of a convex exponential gradient, one-fourth the volume will remain in the mixing chamber.

7. Overlay each gel sandwich with an equal volume of water. For a continuous gel, straighten the pre-positioned combs and insert them fully to form the wells.

8. Following gel casting, pinch off the tubing with the pinch clamp, remove the tubing from the Luer taper coupling, and transfer it to a waste receptacle. Pump out any remaining acrylamide from the chambers and pour rinse water into both the mixing and reservoir chamber. Turn the peristaltic pump on to its maximum velocity to flush out the system. Water should flow from the gradient former through the peristaltic pump and down all the tubing to keep it from being plugged with any polymerized residual acylamide.

Section 6
Gradient Former Care and Maintenance

After use, disassemble the gradient former and rinse all parts with distilled water. If the gel polymerizes in the gradient former during casting or before the system can be flushed with water, the unit can be easily cleaned. The valve stem and stopcock are easily removed. The needle will probably have to be replaced (catalog number 165-2007). The polymerized gel can usually be removed from the tubing, the Luer coupling, and Y-connector with a little effort, or you can replace them with the Tubing Connection Kit (catalog number 165-2008). Use no organic solvents, strong acid solutions, or ethanol in the gradient former.

Section 7
Theory of Linear and Exponential Gradient Gels

7.1 Linear Gradients

Linear gradients are described by the equation:

$C_t = C_M + (C_R - C_M)\, V_t/2V_o$

where C_t = concentration of gradient being delivered at any time,
C_M = initial concentration in mixing chamber,
C_R = concentration in reservoir,
V_t = volume delivered at time t,
V_o = original volume in each chamber.

A typical linear gradient is shown in Figure 7.1. The slope of the gradient is changed by changing the acrylamide concentration in the mixing and/or reservoir chamber.

7.2 Exponential Gradients

Both convex and concave exponential gradients are described by the equation

$C_t = C_R - (C_R - C_M)\, e - V_t/V_M$

where e = natural base (2.718), and
V_M = volume of acrylamide in the mixing chamber; the other symbols are as above.

Figure 7.2 and 7.3 show how a concave gradient is set up in the gradient former, how the gel pore size varies with the length of the gel, and how the % acrylamide varies with the amount of acrylamide delivered.

Figure 7.4 and 7.5 show how a convex gradient is set up in the gradient former, how the gel pore size varies with the length of the gel, and how the % acrylamide varies with the amount of acrylamide delivered.

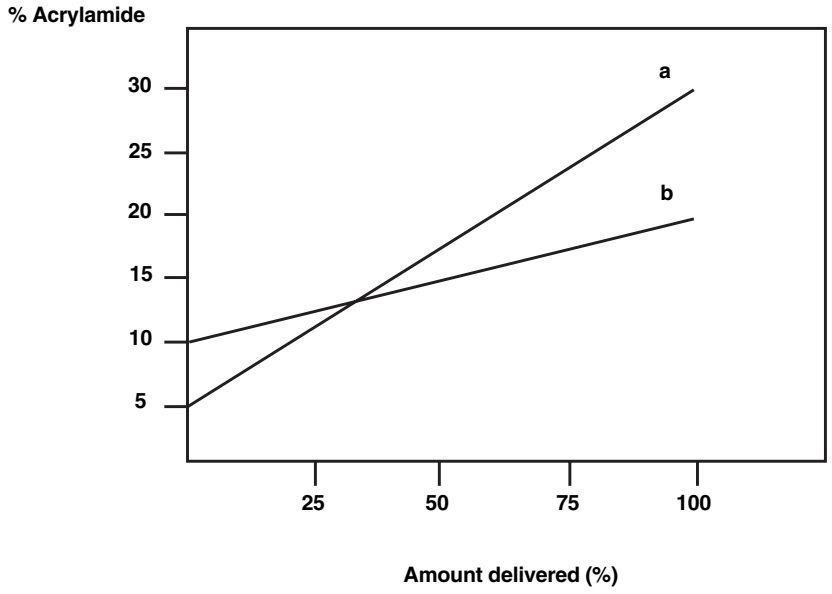

Fig. 7.1. Linear gradient gel. a. 5-30% gradient. b. 10-20% gradient.

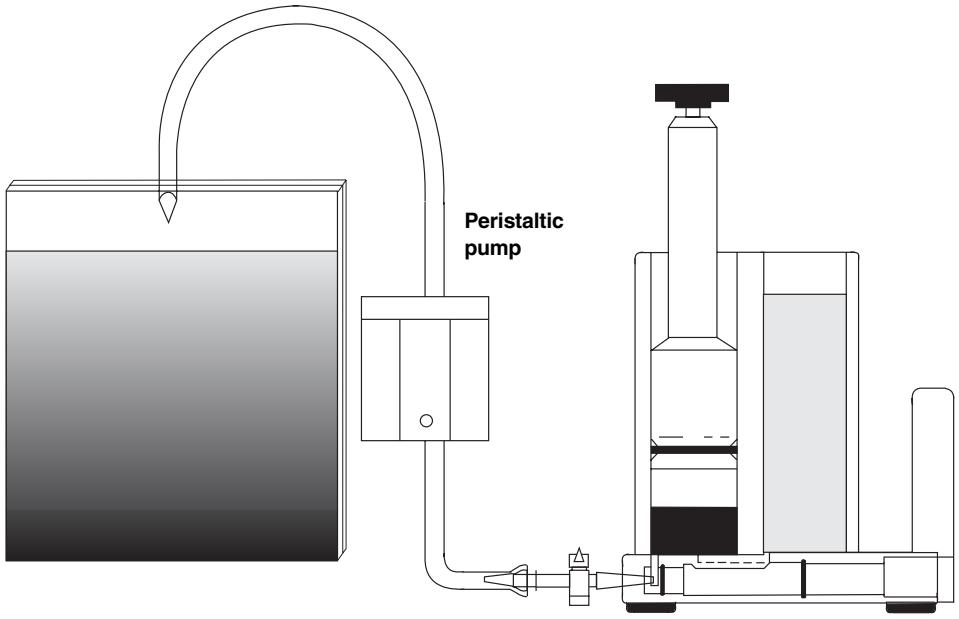

Fig. 7.2. Concave gradient—relation of heavy and light solutions in the mixing and reservoir chambers. Intensity of shading in the slab gel sandwich indicates change in pore size.

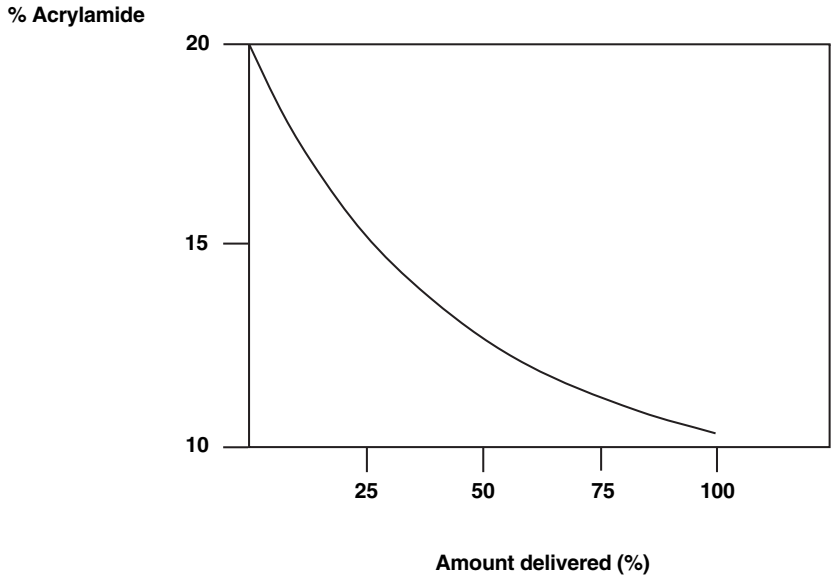

Fig. 7.3. Concave gradient gel.

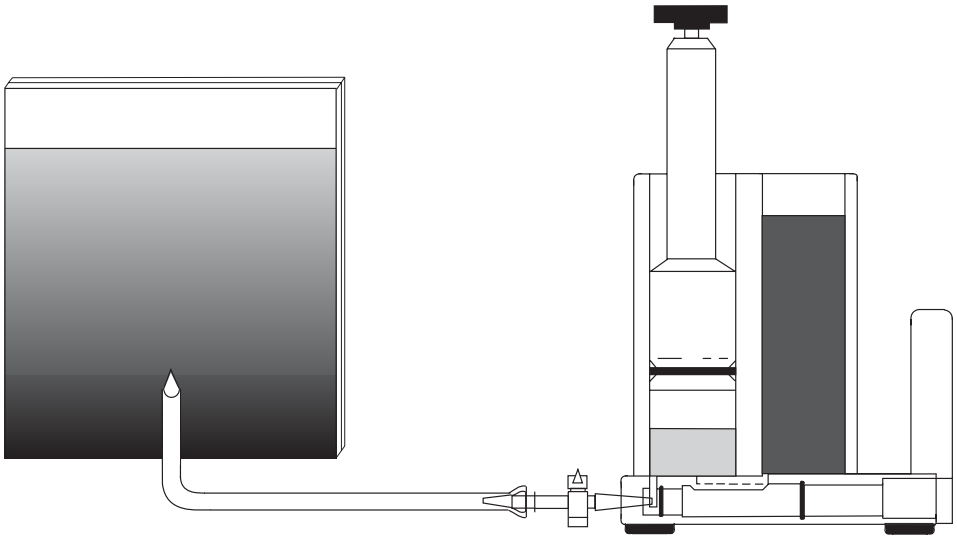

Fig. 7.4. Convex gradient—relation of light and heavy solutions in the mixing and reservoir chambers. Intensity of shading in the slab gel sandwich indicates change in pore size.

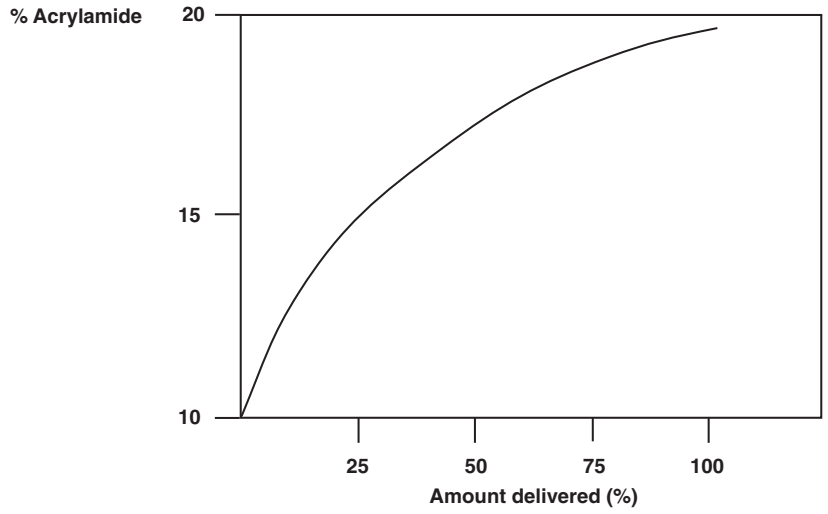

Fig. 7.5. Convex gradient gel.

Section 8
Protocols

8.1 Preparation of Stock Solutions for SDS-PAGE Slab Gels (Laemmli* buffer system)

A. Acrylamide/Bis (30%T, 2. 67%C)

Acrylamide	146.0 g
N'N'-BIS-methylene-acrylamide	4.0 g

(Preweighed Acrylamide/Bis 37.5:1 mixture may be substituted)

Distilled water to 500 ml. Filter and store at 4 °C in the dark. Maximum shelf life under these conditions is 30 days.

B. 1.5 M Tris-HCl, pH 8.8

54.45 g Tris base
150 ml distilled water

Adjust to pH 8.8 with 1 N HCl. Distilled water to 300 ml. Store at 4 °C.

C. 0.5 M Tris-HCl, pH 6.8

6 g Tris base
60 ml distilled water

Adjust to pH 6.8 with HCl. Distilled water to 100 ml. Store at 4 °C.

D. 10% (w/v) SDS

Dissolve 10 g SDS in water with gentle stirring. Distilled water to 100 ml.

E. 10% Ammonium Persulfate (w/v)

100 mg ammonium persulfate. Distilled water to 1 ml. Make fresh daily.

F. Sample Buffer (SDS reducing buffer: 62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 5% b-mercaptoethanol)

Distilled water	4.0 ml
0.5 M Tris-HCl pH 6.8	1.0 ml
Glycerol	0.8 ml
10% SDS	1.6 ml
β-mercaptoethanol	0.4 ml
0.05% (w/v) bromophenol blue (in water)	0.2 ml
	8.0 ml

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

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

G. 5x Electrode (Running) Buffer (25 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.3)

Tris base	45.0 g
Glycine	216.0 g
SDS	15.0 G

Distilled water in 3 L. Do not adjust the pH with acid or base. Store at 4 °C. Warm to 37 °C before use if precipitation occurs. Dilute 300 ml of 5x stock with 1.2 L distilled water to make 1.5 L 1x buffer.

8.2. Calculating Gel Volumes

The total gel volume required is described by the equation:

$$V_T = (W)\,(L)\,(T)\,(N)$$

V_T = Total gel volume; W = Gel width (cm); L = Gel length (cm); T = Gel thickness (cm); N = Number of gel slabs required

Table 8.2 Calculated volumes[a] required per gel for the PROTEAN II xi and Mini-PROTEAN II cells

	PROTEAN II Gel Volume[b]		Mini-PROTEAN II GelVolume[b]
Spacer Thickness	**16 cm length**	**20 cm length**	**7 cm length**
0.50 mm	12.8 ml	16.0 ml	2.9 ml
0.75 mm	19.2 ml	24.0 ml	4.4 ml
1.00 mm	25.6 ml	32.0 ml	5.8 ml
1.50 mm	38.4 ml	48.0 ml	8.8 ml
3.00 mm	76.8 ml	96.0 ml	

Note: With a multi-gel casting chamber, the total gel volume required depends on several factors, including gel thickness, number of gels, gel length, and amount of space-filling. To make a precise determination of the volume required for a particular application, set up the casting chamber with the glass plates, spacers, and combs to form the required number of gel sandwiches and measure the volume of deionized distilled water required to fill it to the desired level. Then disassemble the chamber and rinse and dry the parts.

a. The volumes reported completely fill the gel sandwich to the top of the inner plate. The amount of separating gel may be adjusted depending on application (with or without comb, with or without stacking gel, etc.).

b. The total volume required for multiple gels can be determined by multiplying the volume of a single gel by the number of gels required.

8.3 Gel Preparation

Monomer concentrations as high as 22% T can be prepared from a 30% T stock solution as presented in Table 8.3. When monomer concentrations greater than 22% T are required, the Acrylamide/Bis stock solutions must be increased to 40% T 0r 50% T.

Table 8.3. Formulations for SDS-PAGE Separating and Stacking Gels

	Separating Gel (0.375 M Tris, pH 8.8)				Stacking Gel (125 M Tris, pH 6.8)
Monomer Concentration (%T, 2.67%C)[C]	10%	20%	X%(<15%)	X%>(15%)	4.0%
Acrylamide/Bis (30%T, 2.67%C) Stock	33.3 ml	66.6 ml	d ml	d ml	1.3 ml
Distilled water	40.2 ml	6.9 ml	e ml	e ml	6.1 ml
1.5 M Tris-HCl, pH 8.8	25.0 ml	25.0 ml	25.0 ml	25.0 ml	
.5 M Tris-HCl, pH 6.8					2.5 ml
10% (w/v) SDS	1.0 ml	1.0 ml	1.0 ml	1.0 ml	100 ml
10% ammonium persulfate (fresh)[f]	500 µl	500 µl	500 µl	500 µl	50 µl
TEMED[f] (monomer concentration <15%)	50 µl		50 µl		10 µl[f]
TEMED [f,g] (monomer concentration >15%)		25 µl		25 µl	
TOTAL MONOMER [h]	100 ml	100 ml	100 ml	100 ml	10 ml

c. The pore size of the polyacrylamide gel can be changed by adjusting either the total monomer concentration (%T) or by adjusting the crosslinking monomer concentration (%C). The most common method of changing the pore size is to adjust the total monomer concentration (%T). In diluting a stock solution, the crosslinking monomer concentration (%C) is independent of the final total monomer concentration (%T).

%T=[g Acrylamide + g Bis-Acrylamide/Total Volume] x 100
%C=[g Bis-Acrylamide/(g Acrylamide + g Bis-Acrylamide)] x 100

d. Calculate the volume of Acrylamide/Bis stock required for the desired total monomer concentration with the following formula: volume 30% T, 2.67% C Stock = (X % T) x (3.33). For example, the volume of Acylamide/Bis stock required to prepare 100 ml final volume of 10% T monomer solution would be 33.0 ml; 10 x 3.33 = 33.3 ml.

e. Calculate the volume of water required for the desired total monomer concentration with the following formula: volume water = 73.5 - (volume 30% T, 2.67% C stock used). For example, the volume of water required to prepare 100 ml final volume of a 10% T monomer solution would be 40.4 ml: 73.5 - 33.3 ml = 40.2 ml.

f. Prepare the monomer solution by combining all reagents except ammonium persulfate and TEMED. Dearate the solution under vacuum for at least 15 minutes. Add the two catalysts just prior to casting the gels.

g. Higher concentrations of monomer polymerize more evenly with lower concentrations of TEMED.

h. One can prepare any desired volume of monomer solution by multiplying to 100 ml recipe by the desired multiplying factor.

i. Higher TEMED concentrations and faster polymerization are required for the stacking gel because of the inhibitory effect of atmospheric oxygen associated with the comb.

8.4 Linear Gradient Gels

For linear gradients, the volume in the mixing chamber (V_m) equals the volume in the reservoir chamber (V_r). Try volume in each chamber is one-half of the total gel volume (V_T):

$$V_T = V_m + V_r \qquad \text{or } V_m = V_r = 0.5\ V_T$$

First calculate the total gel volume required. Then determine the amount needed for the mixing and reservoir chambers. It is easier to prepare a volume slightly more than the required gel volume and then quantitatively transfer the required volume into mixing or reservoir chamber.

Example 1: Describe how to prepare a linear 10-20% gradient for casting six 16 x 20 x 0.15 cm gels in a PROTEAN II multi-casting chamber. The slab gels will be used for 2-D work and a stacking gel is not required.

1. When a gel casting chamber is used, the gradient must be poured by bottom filling. Therefore, the mixing chamber of the Model 395 Gradient Former must contain the lighter (10%) monomer solution and the reservoir chamber must contain the heavier (20%) monomer solution.

2. When using a multi-gel casting chamber, the total gel volume required depends on several factors, including gel thickness, number of gels, gel length, and amount of space-filling. To precisely determine the volume required for six 16 x 20 x 0.15 cm gels, set up the casting chamber with he glass plates, spacers and the necessary space fillers and measure the volume of deionized distilled water required for a 20 cm length. In this example, let us assume that the total gel volume needed is 310 ml (your actual required gel volume may differ slightly from this hypothetical value). Disassemble the chamber and rinse and dry the parts.

3. Reassemble the multi-gel chamber as described in step 2.

4. The amount of monomer required for each chamber will be 155 ml (310/2). It is best to prepare 170 ml each of a 10% and 20% monomer solution and to quantitatively transfer 155 ml of each solution into their respective chambers. The formulation for each monomer solution can be calculated using Table 8.2 and a multiplying factor of 1.7 (170 ml required volume/100 ml volume in Table 8.2).

Monomer Concentration	10%	20%
30% stock	56.6 ml	113.2 ml
Distilled water	68.3 ml	11.7 ml
1.5 M Tris-HCl, pH 8.8	42.5 ml	42.5 ml
10% (w/v) SDS	1.7 ml	1.7 ml
Degas		
10% ammonium persulfate	850 µl	850 µl
TEMED	85 µl	42.5 µl
Total monomer volume	170 ml	170 ml

155 ml of the 10% solution will be transferred to the mixing chamber and 155 ml of the 20% solution will be transferred to the reservoir chamber.

5. Set up and cast the gradient as outlined in Section 5, steps 4-8. Overlay the gels with an equal volume of water.

8.5 Exponential Gradient Gels

For an exponential gradient gel, the volume in the mixing chamber is one fourth the total required gel volume (0.25 V_T) and the volume in the reservoir chamber equals the total required gel volume (1.0 V_T).

First calculate the total gel volume required. Then determine the amount needed for the mixing and reservoir chambers. It is easier to prepare a volume slightly more than the required gel volume and then quantitatively transfer the required volume into mixing or reservoir chambers.

Example 2: How to prepare a concave 5-15% exponential gradient for one 16 x 16 x 0.15 cm gel with a 2 cm stacking gel to be cast in the PROTEAN II xi casting stand.

1. A concave exponential gradient must be cast from the top. The heavier 15% monomer will be placed in the mixing chamber and the lighter 5% monomer will be placed in the reservoir chamber of the Model 385 Gradient Former.

2. From Table 8.1, 38.4 ml total gel volume is required. 9.6 ml (0.25 x 38.4 or 0.25 V_T) of the 15% monomer solution is required for the mixing chamber and 38.4 ml (V_T) of the 5% monomer solution is required for the reservoir chamber. It is convenient to prepare 15 ml of the 15% solution and 50 ml of the 5% solution and quantitatively transfer the required volumes into the respective chamber.

3. The multiplying factor for the 15% monomer solution is 0.15 (15/100) and the multiplying factor for the 5% monomer solution is 0.5 (50/100). Prepare the gel solution using Table 8.2:

Monomer Concentration	5%	15%
30% stock	8.3 ml	7.5 ml
Distilled water	28.4 ml	3.5 ml
1.5 M Tris-HCl, pH 8.8	12.5 ml	3.8 ml
10% (w/v) SDS	.5 ml	50 µl
Degas		
10% ammonium persulfate	250 µl	75 µl
TEMED	25 µl	7.5 µl
Total monomer volume	50 ml	15 ml

9.6 ml of the 15% solution will be transferred to the mixing chamber and 38.4 ml of the 5% solution will be transferred to the reservoir chamber.

4. Set up and pour the gradient as outlined in Section 3.1, steps 2-8. Overlay the gels with water saturated isobutanol. After the separating gel has polymerized, remove the water saturated isobutanol and wash the top of the gel with distilled deionized water. Prepare the stacking gel as described in Table 8.2. Cast the stacking gel and insert the comb to form the wells.

Section 9
Equipment and Accessories

9.1 Model 385 and Model 395 Gradient Formers and Accessories

Product Description	Catalog Number
Model 385 Gradient Former, includes main body assembly with valve stem, and tubing connection kit (stopcock, Luer taper coupling, Y-connector, and tubing)	165-2000
Model 395 Gradient Former, includes main body assembly with valve stem, and tubing connection kit (stopcock, Luer taper coupling, Y-connector, and tubing)	165-2001
Exponential Piston, Model 395	165-2005
Exponential Piston, Model 385	165-2006
Gradient Pouring Needles for Model 385 and 395, 2	165-2007
Tubing Connection Kit (stopcock, Luer taper coupling, tubing (1/8" ID, 3 ft), and Y-connector)	165-2008

9.2 Additional Required Equipment (not included)

Peristaltic pump capable of delivering 10 ml/min for Model 385 Gradient Former
Peristaltic pump capable of delivering 100 ml/min for Model 395 Gradient Former
1" stir bar for Model 385 Gradient Former
2" stir bar for Model 395 Gradient Former
Magnetic stirring motor

9.3 PROTEAN II xi and Mini-PROTEAN II Vertical Electrophoresis Cells and Accessories

Product Description	Catalog Number
PROTEAN II xi 16 cm Slab Cell*	165-1801
1.5 mm, 15 well	165-1802
1.0 mm, 15 well	165-1803
0.75 mm, 15 well	165-1804
PROTEAN II xi 20 cm Slab Cell*	165-1811
1.5 mm, 15 well	165-1812
1.0 mm, 15 well	165-1813
0.75 mm, 15 well	165-1814

All PROTEAN II xi slab cells include the central cooling core with gaskets and core caps, lower buffer chamber, lid with power cables, 2 sets of glass plates, 4 sandwich clamps, 2 combs, an upper buffer dam, a casting stand with gaskets, a leveling bubble, PAGE starter kit, and instructions.

* Cells marked with (*) contain all of the above except spacers and combs (order separately).

Product Description	Catalog Number
PROTEAN II xi 2-D Cell, 1.0 mm, 16 cm	165-1931
PROTEAN II xi 2-D Cell, 1.5 mm, 16 cm	165-1932
PROTEAN II xi 2-D Cell, 1.0 mm, 20 cm	165-1933
PROTEAN II xi 2-D Cell, 1.5 mm, 20 cm	165-1934

PROTEAN II xi 2-D cells include central cooling core, lower buffer chamber, lid with power cables, 2 sets of glass plates (with bevel), 4 sandwich clamps, 24 glass tubes, 2 tube cell adapters, 16 stoppers, 48 grommets (4-8 mm O.D. tubes), 2 2-D combs, 4 spacers, 1 upper buffer dam, slab casting stand, leveling bubble, PAGE starter kit, and instructions.

Product Description	Catalog Number
PROTEAN II xi Multi-Cell, includes 3 central cooling cores with gaskets, tank, lid with power cables, 1 upper buffer dam, PROTEAN II xi Multi-Gel casting chamber with accessories, leveling bubble, and instructions (order spacers, plates, clamps and combs separately)	165-1951
PROTEAN II xi Multi-Gel Casting Chamber, includes casting chamber, sealing plate Luer taper connector, silicone gasket, package of 15 separation sheets, set of 4 acrylic blocks, and instructions	165-2025

PROTEAN II xi and PROTEAN II xi Multi-Cell Glass Plates

	16 cm cell	20 cm cell
Inner Plate (shorter), 2	165-1821	165-1823
Outer Plate (longer), 2	165-1822	165-1824
Frosted Inner Plate, 2 (agarose gels)*	165-1825	165-1826
Beveled Inner Plates, 2 (2-D procedures)*	165-1827	165-1828

* Used in conjunction with regular outer plate.

Protean II xi Cell Spacers (set of 4)

	0.5 mm	0.75 mm	1.0 mm	1.5 mm	3.0 mm
16 cm	165-1841	165-1842	165-1843	165-1844	165-1845
20 cm	165-1846	165-1847	165-1848	165-1849	165-1850

PROTEAN II xi Cell PTFE Combs

	0.5 mm	0.75 mm	1.0 mm	1.5 mm	3.0 mm
25 well	—	165-1861	165-1862	165-1863	—
20 well	165-1865	165-1866	165-1867	165-1868	165-1869
15 well	165-1870	165-1871	165-1872	165-1873	165-1874
10 well	165-1875	165-1876	165-1877	165-1878	165-1879
5 well	—	165-1881	165-1882	165-1883	165-1884
3 well	—	—	165-1887	165-1888	165-1889
Blank	165-1890	165-1891	165-1892	165-1893	165-1894
2-D (1 ref well)-	—	165-1897	165-1898	165-1899	

Product Description	Catalog Number
Mini-PROTEAN II Slab Cell, 10 well combs, 0.75 mm spacers, includes inner cooling core with gaskets, lower buffer chamber, lid with power cables, 3 sets of glass plates, 2 clamp assemblies, 10 well, 0.75 mm thick combs (2), 0.75 mm thick spacers (4), casting stand with gaskets, leveling bubble, PAGE starter kit, and instructions	165-2940
Mini-PROTEAN II Slab Cell, same as 165-1940 without comb and spacers	165-2941
Mini-PROTEAN II Multi-Casting Chamber, includes casting chamber body with front clamp screws, cover with gasket, 10 sets of glass plates, 15 separation sheets, set of 4 acrylic blocks, stopcock, Luer fitting, and instructions	165-2950
Mini-PROTEAN II Glass Plates, 10 sets, a set consists of 1 shorter (inner) plate and 1 longer (outer) plate	165-2912

Mini-PROTEAN II PTFE Combs

2-D/Preparative

	5 well	10 well	15 well	(1 Reference Well)
0.5 mm	165-1915	165-2919	165-2923	—
0.75 mm	165-2916	165-2920	165-2924	165-2927
1.0 mm	165-2917	165-2921	165-2925	165-2928
1.5 mm	165-2918	165-2922	165-2926	165-2929

Mini-PROTEAN II Spacers (set of 4)

0.5 mm	0.75 mm	1.0 mm	1.5 mm
165-2930	165-2931	165-2932	165-2933

9.4 Electrophoresis Chemicals

Acrylamide, 99.9%	100 g	161-0100
	500 g	161-0101
	1 kg	161-0107
	2 kg	161-0103
Preweighed Acrylamide/Bis, 37.5:1 mixture	30 g	161-0122
	150 g	161-0125
Preweighed Acrylamide/Bis. 29.5:1 mixture	30 g	161-0121
	150 g	161-1024
Preweighed Acrylamide/Bis, 19:1 mixture	30 g	161-0120
	150 g	161-0123
Bis (N, N'-methylene-bis-acrylamide)	5 g	161-0200
	50 g	161-0201
Tris	500 g	161-0716
	1 kg	161-0719
Glycine	250 g	161-0717
	1 kg	161-0718

Product Description		Catalog Number
SDS (sodium dodecylsulfate)	25 g	161-0300
	100 g	161-0301
	1 kg	161-0302
Boric Acid	500 g	161-0750
	1 kg	161-0751
Ammonium Persulfate	10 g	161-0700
TEMED	5 ml	161-0800
	50 ml	161-0801
Dithiothreitol	1 g	161-0610
	5 g	161-0611
2-mercaptoethanol	25 ml	161-0710
SDS-PAGE Standards, 10,000-100,000 MW		161-0304
SDS-PAGE Standards, 40,000-250,000 MW		161-0303
Pre-stained SDS-PAGE Standards, 15,000-100,000 MW		161-0305
Biotinylated SDS-PAGE Standards, Low Range		161-0306
Biotinylated SDS-PAGE Standards, High Range		161-0311
Biotinylated SDS-PAGE HMW Standards Kit, Avidin HRP		161-0312
Biotinylated SDS-PAGE LMW Standards Kit, Avidin HRP		161-0307
Biotinylated SDS-PAGE HWM Standards Kit, Avidin AP		161-0313
Biotinylated SDS-PAGE LMW Standards Kit, Avidin AP		161-0308
Silver Stain Kit, includes 1 bottle oxidizer concentrate, 1 bottle silver reagent concentrate and 4 bottle developer. Enough to stain approximately 24 gels.		161-0443
Coomassie Blue R-250	10 g	161-0400
Bromophenol Blue	10 g	161-0404
Triton X-100	500 ml	161-0407
CHAPS	1 g	161-0460
CHAPSO	1 g	161-0465
Urea	250 g	161-0730
	1 kg	161-0731

BIO-RAD

Bio-Rad Laboratories

ISO 9001 registered | www.bio-rad.com

Life Science Group

Bio-Rad Laboratories Main Office 2000 Alfred Nobel Drive, Hercules, California 94547, Ph. (510) 741-1000, Fx. (510)741-5800
Also in: Australia Ph. 02-9914-2800, Fx. 02-9914-2889 **Austria** Ph. (1)-877 89 01, Fx. (1) 876 56 29 **Belgium** Ph. 09-385 55 11, Fx. 09-385 65 54
Canada Ph. (905) 712-2771, Fx. (905) 712-2990 **China** Ph. (86-10) 2046622, Fx. (86-10) 2051876 **Denmark** Ph. 39 17 9947, Fx. 39 27 1698
Finland Ph. 90 804 2200, Fx. 90 804 1100 **France** Ph. (1) 43 90 46 90, Fx. (1) 46 71 24 67 **Germany** Ph. 089 31884-0, Fx. 089 31884-100
Hong Kong Ph. 7893300, Fx. 7891257 **India** Ph. 91-11-461-0103, Fx. 91-11-461-0765 **Israel** Ph. 03 951 4127, Fx. 03 951 4129
Italy Ph. 02-21609.1, Fx. 02-21609.399 **Japan** Ph. 03-5811-6270, Fx. 03-5811-6272 **The Netherlands** Ph. 0313 18-540666, Fx. 0313 18-542216
New Zealand Ph. 09-443 3099, Fx. 09-443 3097 **Singapore** Ph. (65) 272-9877, Fx. (65) 273-4838 **Spain** Ph. (91) 661 70 85, Fx. (91) 661 96 98
Sweden Ph. 46 (0) 8 627 50 00, Fx. 46 (0) 8 627 54 00 **Switzerland** Ph. 01-809 55 55, Fx. 01-809 55 00
United Kingdom Ph. 0800 181134, Fx. 01442 259118

M1652000 Rev C