Model 485
Gradient Former

Instruction Manual

Catalog Number
165-4120

Table of Contents

Section 1
Introduction

The Model 485 Gradient Former is primarily used to construct reproducible linear and exponential polyacrylamide gradient gels. It has a 175 ml capacity, making it ideal for preparation of up to 12 identical Mini-PROTEAN® 3 gradient gels. It can also be used to prepare PROTEAN II gels.

The mixing (labeled "light") and reservoir (labeled "heavy") chambers have identical dimensions. The outlet from the mixing chamber leads into a multi-casting chamber. The exponential piston is available as an accessory; it is used to fix the volume in the mixing chamber to produce concave or convex exponential gradients.

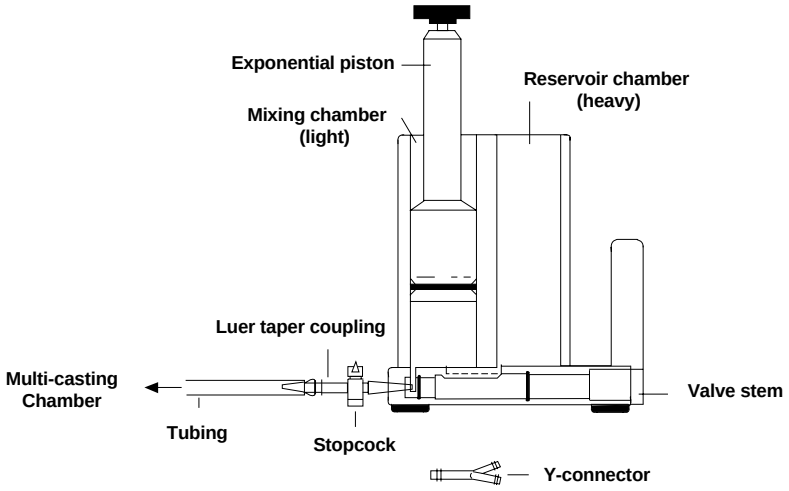

Figure 1.1 Model 485 Gradient Former, major parts

1.1 Specifications

Overall size	10 cm x 10 cm x 16 cm (W x L x H)
Weight	500 g
Capacity	40–175 ml
Materials of construction	
Body	Machined from an acrylic block
Valve stem	Delrin®
Stopcock	Plastic
Piston	Delrin
Tubing	Tygon® 1/8” ID

Section 2
Pouring Multiple Gradient Gels in the Multi-Casting Chamber

Note: This procedure can be used to produce gels with either a linear or a convex exponential gradient. Cast a minimum of 4 Mini-PROTEAN 3 gels using this procedure.

An inexperienced user should practice all steps ahead of time so that the procedure is completed quickly. The best guarantee of reproducibility from gradient to gradient is careful technique on the part of the operator.

Wear rubber gloves to prevent exposure to unpolymerized acrylamide, a neurotoxin.

2.1 Calculate the Volume of Acrylamide to Prepare

The first time gels of a certain thickness are cast, it is necessary to empirically determine the required volume of acrylamide. If you are using the Mini-PROTEAN 3 multi-casting chamber, assemble the stack of Mini-PROTEAN 3 sandwiches (as described in the multi-casting chamber manual) and inject a measured volume of water through the stopcock. You will prepare this volume (+5 ml) of acrylamide. (A minimum working volume of 40 ml is required for the gradient former.) Disassemble the chamber and dry all components.

See Appendix A for additional information.

2.2 Calculate the Chamber Volumes

To create a linear gradient, the volume in each chamber is ½ the total gel volume required (or 20 ml, whichever is greater.)

For a convex exponential gradient, the light solution in the mixing chamber will be ¼ the total gel volume (minimum 10 ml) and the heavy solution in the reservoir chamber will be equal to the total gel volume (minimum 40 ml).

Combine all reagents except the initiators (usually APS and TEMED), and degas each solution under vacuum for at least 15 minutes.

2.3 Reassemble the Multi-Casting Chamber

Refer to the Mini-PROTEAN 3 multi-casting chamber instruction manual. Be certain that all of the parts are clean and dry.

2.4 Pour the Gels

Note: If the flow rate is kept constant, the gradients will be reproducible. Tubing size, the volume of acrylamide in the chambers, and the rate of stirring must be kept constant. If the rate of stirring changes, then the amount of acrylamide pulled into the front chamber from the back chamber when the connection between the two chambers is opened will vary. This will change the gradient.

2.4.1 To Pour Linear Gradient Gels

a. Arrange the tubing so that there is as short a distance as possible between the stopcock opening and the inlet to the gel casting chamber.

Note: The level of the gradient former stopcock must be placed above the top of the gel sandwich. This creates a hydrostatic head large enough to pour the gels within 10 minutes from the time the initiators are added to the light solution. To create uniform gradients, all of the acrylamide must be in the gel sandwich before polymerization begins.

b. Add a 1" stirbar in the mixing chamber (with the valve stem closed.) The mixing chamber is labeled "light". Place the gradient former on a magnetic stir plate.

c. Add the initiators to the light solution, swirl it 8 to 10 times, and pour the mixture into the mixing chamber. This is the start of the 10 minutes.

d. Add the initiators to the heavy solution, swirl it 8 to 10 times, and pour it into the reservoir chamber. Start the stir plate and adjust the speed so that you get good mixing; the bottom level of the vortex should be nearly the same as the acrylamide level in the reservoir chamber.

e. Quickly open the valve stem and stopcock. The acrylamide will flow down the tubing to the gel sandwich. Do not allow air bubbles to enter the gel. Overlay the acrylamide with water or water-saturated isobutanol. **Note**: each gel must have the same volume of overlay solution. For a continuous buffer system, insert a comb at an angle (this reduces the chance of bubble formation.)

f. Close the stopcock on the multi-casting chamber after the gels are cast. Remove the tubing. Immediately flush the system with water to prevent polymerization of residual acrylamide within the gradient former.

2.4.2 To Pour Convex Exponential Gradient Gels

a. Arrange the tubing so that there is as short a distance as possible between the stopcock opening and the inlet to the gel casting chamber.

Note: The level of the gradient former stopcock must be placed above the top of the gel sandwich. This creates a hydrostatic head large enough to pour the gels within 10 minutes from the time the initiators are added to the light solution. To create uniform gradients, all of the acrylamide must be in the gel sandwich before polymerization begins.

b. Add a 1" stirbar in the mixing chamber (with the valve stem closed.) The mixing chamber is labeled "light". Place the gradient former on a magnetic stir plate.

c. Add the initiators to the light solution, swirl 8 to 10 times, and pour into the mixing chamber, leaving the valve stem closed. This is the start of the 10 minutes.

d. Immediately fix the volume with the exponential piston 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.

e. Add the initiators to the heavy solution, swirl it 8 to 10 times, and pour it into the reservoir chamber. Start the stir plate and adjust the speed so that you get good mixing; the bottom level of the vortex should be nearly the same as the acrylamide level in the reservoir chamber.

f. Quickly open the valve stem and stopcock. The acrylamide will flow down the tubing to the gel sandwich. Do not allow air bubbles to enter the gel. Overlay the acrylamide with water or water-saturated isobutanol. **Note**: each gel must have the same volume of overlay solution. For a continuous buffer system, insert a comb at an angle (this reduces the chance of bubble formation.)

g. Close the stopcock on the multi-casting chamber after the gradient is cast. Remove the tubing. Immediately flush the system with water to prevent polymerization of residual acrylamide within the gradient former.

Section 3
Pouring One Large-Format Gradient Gel

Pouring a single Mini-PROTEAN 3 gradient gel is not recommended. The minimum volume for the gradient former is 40 ml, and you should use nearly all of the solution in pouring a gradient gel. *These instructions are intended for casting large-format polyacrylamide gels*.

An inexperienced user should practice all steps ahead of time so that the procedure is completed quickly. The best guarantee of reproducibility from gradient to gradient is careful technique on the part of the operator.

Wear rubber gloves to prevent exposure to unpolymerized acrylamide, a neurotoxin.

Note: If the rate of flow is kept constant, then the gradients will be reproducible. Tubing size, the needle size and size of the hole in the needle, the volumes in the chambers, and the rate of stirring of the stir plate must be kept constant. If the rate of stirring changes, then the amount of acrylamide pulled into the front chamber from the back chamber when the connection between the two chambers is opened will vary. This will change the gradient.

3.1 Calculate the Volume of Acrylamide to Prepare

The first time gels of a certain thickness are cast, it is necessary to empirically determine the required volume of acrylamide. Inject a measured volume of water into the gel sandwich. You will prepare this volume (+5 ml) of acrylamide. (A minimum working volume of 40 ml is required for the gradient former.) Disassemble the chamber and dry all components.

3.2 Calculate the Chamber Volumes

To create a linear gradient, the volume in each chamber is ½ the total gel volume required (or 20 ml, whichever is greater.)

For a convex exponential gradient, the light solution in the mixing chamber will be ¼ the total gel volume (minimum 10 ml) and the heavy solution in the reservoir chamber will be equal to the total gel volume (minimum 40 ml).

For a concave exponential gradient, the heavy solution in the mixing chamber will be ¼ the total gel volume (minimum 10 ml) and the light solution in the reservoir chamber will be equal to the total gel volume (minimum 40 ml).

Combine all reagents except the initiators (usually APS and TEMED), and degas each solution under vacuum for at least 15 minutes.

3.3 Assemble the Glass Plate Sandwich

Set up the glass plate for casting (as indicated in the electrophoresis cell instruction manual.)

3.4 Pour the Gel

Note: The level of the gradient former stopcock must be placed above the top of the gel sandwich. This creates a hydrostatic head large enough to pour the gel within 10 minutes from the time the initiators are added to the light solution. To create uniform gradients, all of the acrylamide must be in the gel sandwich before polymerization begins.

3.4.1 Pour a Linear or Concave Gradient 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**: For this application, the heavy solution will be poured in the mixing chamber labeled "light", and the light solution will be poured in the mixing chamber labeled "heavy".

a. Arrange the tubing so that there is as short a distance as possible between the stopcock opening and the needle at the end of the tubing. (Alternatively, you can cut the end of the tubing at an angle.) The end of the needle or tubing will lie against the glass plate while the acrylamide is being poured into the sandwich.

b. Place a 1" stirbar in the mixing chamber (with the valve stem closed.) The mixing chamber is labeled "light".

c. Add the initiators to the light solution, swirl it 8 to 10 times, and pour the mixture into the reservoir chamber (which is labeled "heavy"). This is the start of the 10 minutes. Leave the valve stem closed.

d. Add the initiators to the heavy solution, swirl it 8 to 10 times, and pour it into the mixing chamber (which is labeled "light"). Start the stir plate and adjust the speed so that you get good mixing; the bottom level of the vortex should be nearly the same as the acrylamide level in the reservoir chamber.

For a concave gradient, insert the exponential piston into the mixing chamber to 1 cm above the level of the acrylamide, tighten the screw top handle to hold the piston in place, and then start the stir plate.

e. Quickly open the valve stem and stopcock. The acrylamide will flow through the tubing and needle to the gel sandwich. Do not allow air bubbles to enter the gel. Overlay the acrylamide with water or water-saturated isobutanol. For a continuous buffer system, insert a comb. (The comb should be inserted at an angle to prevent the formation of bubbles.)

f. Remove the tubing and disassemble the gradient former. Immediately flush the system with water to prevent polymerization of residual acrylamide within the gradient former.

3.4.2 Pour a Linear or Convex Gradient Gel from the Bottom

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

An inexperienced user should practice all steps ahead of time so that the procedure is completed quickly. The best guarantee of reproducibility from gradient to gradient is careful technique on the part of the operator.

Wear rubber gloves to prevent exposure to unpolymerized acrylamide, a neurotoxin.

a. Arrange the tubing so that there is as short a distance as possible between the stopcock opening and the needle at the end of the tubing. Insert the needle into the gel sandwich through the gasket on the bottom of the casting stand.

b. Place a 1" stirbar in the mixing chamber (with the valve stem closed.) The mixing chamber is labeled "light".

c. Add the initiators to the light solution, swirl it 8 to 10 times, and pour the mixture into the mixing chamber (which is labeled "light"). This is the start of the 10 minutes. Leave the valve stem closed.

For a convex gradient, insert the exponential piston into the mixing chamber to 1 cm above the level of the acrylamide, tighten the screw top handle to hold the piston in place.

d. Add the initiators to the heavy solution, swirl it 8 to 10 times, and pour it into the reservoir chamber (which is labeled "heavy"). Start the stir plate and adjust the speed so that you get good mixing; the bottom level of the vortex should be nearly the same as the acrylamide level in the resevoir chamber.

e. Quickly open the valve stem and stopcock. The acrylamide will flow through the tubing and needle to the gel sandwich. Do not allow air bubbles to enter the gel. Overlay the acrylamide with water or water-saturated isobutanol. For a continuous buffer system, insert a comb. (The comb should be inserted at an angle to prevent the formation of bubbles.)

f. Remove the tubing and disassemble the gradient former. Immediately flush the system with water to prevent polymerization of residual acrylamide within the gradient former.

Section 4
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, remove the valve stem and stopcock and rinse thoroughly. The needle can be replaced (catalog number 165-2007). A Tubing Connection Kit (catalog number 165-2008) includes replacement Tygon tubing, a luer fitting, stopcock, and a Y connector. Do not use organic solvents, strong acid solutions or ethanol in the gradient former.

Section 5
Product Information

Catalog Number	Description
165-4120	**Model 485 Gradient Former**
165-2006	**Exponential Piston**
165-2007	**Gradient Pouring Needles**, 2
165-2008	**Tubing Connection Kit**, includes stopcock, tapered luer coupling, tubing (1/8 inch ID, 3 feet), and Y-connector

Appendix A
Estimated Volume of Acrylamide for 12 Mini-PROTEAN 3 Gels

The first time gels of a certain thickness are cast, it is necessary to empirically determine the required volume of acrylamide. Assemble the stack, and inject a measured volume of water through the stopcock. Prepare this volume (+5 ml) of acrylamide.

As a guideline, this is the estimated volume of acrylamide for 12 Mini-PROTEAN 3 gels:

Spacer plates	Volume for 12 gels	Volume to prepare
0.5 mm	70 ml	75–80 ml
0.75 mm	80 ml	85–90 ml
1.0 mm	100 ml	105–110 ml
1.5 mm	140 ml	145–150 ml

Sample calculation: preparing twelve 1.0 mm 4–20% gradient gels:

Casting twelve 1.0 mm gels requires 100 ml; prepare 110 ml.

Divide the total volume by 2 to get the volume required for each chamber. (For this example, make 55 ml for the light chamber and 55 ml for the heavy chamber.)

Solution Volume Calculations:

Light Solution (4%)

Acrylamide
30% stock solution
(30%) (**X** ml) = (4%) (55 ml) **X** = 7.3 ml

Tris-Cl Buffer
1.5M Tris-Cl stock buffer pH 8.8
(1.5M) (**X** ml) = (.375M) (55 ml) **X** = 13.8 ml

Water
(55 ml)–(7.3 ml + 13.8 ml) = **X** **X** = 34 ml

APS
10% Stock solution
(500 µl) / (100 ml) = (**X** µl) / (55 ml) **X** = 275 µl

TEMED
10% of the APS volume; (275 µl) / 10 = **X** **X** = 27.5 µl

Heavy Solution (20%)

Acrylamide
30% Stock solution
(30%) (**X** ml) = (20%) (55 ml) **X** = 36.7 ml

Tris-Cl Buffer
1.5M Tris-Cl Stock Buffer pH 8.8
(1.5M) (**X** ml) = (.375M) (55 ml) **X** = 13.8 ml

Water
(55 ml)–(36.7 ml + 13.8 ml) = **X** **X** = 4.5 ml

APS
(500 µl) / (100 ml) = (X µl) / (55 ml) **X** = 275 µl

TEMED
10% the APS volume; (275 µl) / 10 = **X** **X** = 27.5 µl

Bio-Rad Laboratories

Life Science Group

Website *www.bio-rad.com* ***Bio-Rad Laboratories Main Office*** *2000 Alfred Nobel Drive, Hercules, CA 94547, Ph. (510) 741-1000, Fx. (510)741-5800*
Also in: Australia *Ph. 02 9914 2800, Fx. 02 9914 2889* ***Austria*** *Ph. (01) 877 89 01, Fx. (01) 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. 86-10-62051850/51, Fx. 86-10-62051876* ***Denmark*** *Ph. 45 39 17 99 47, Fx. 45 39 27 16 98*
Finland *Ph. 358 (0)9 804 2200, Fx. 358 (0)9 804 1100* ***France*** *Ph. 01 43 90 46 90, Fx. 01 46 71 24 67* ***Germany*** *Ph. 089 318 84-0, Fx. 089 318 84-100*
Hong Kong *Ph. 852-2789-3300, Fx. 852-2789-1257* ***India*** *Ph. (91-11) 461-0103, Fx. (91-11) 461-0765* ***Israel*** *Ph. 03 951 4127, Fx. 03 951 4129*
Italy *Ph. 39-02-216091, Fx.39-02-21609-399* ***Japan*** *Ph. 03-5811-6270, Fx. 03-5811-6272* ***Korea*** *Ph. 82-2-3473-4460, Fx. 82-2-3472-7003*
Latin America *Ph. 305-894-5950, Fx. 305-894-5960* ***Mexico*** *Ph. 514-2210, Fx. 514-2209* ***The Netherlands*** *Ph. 0318-540666, Fx. 0318-542216*
New Zealand *Ph. 64-9-4152280, Fx. 64-9-4152284* ***Norway*** *Ph. 22-74-18-70, Fx. 22-74-18-71* ***Russia*** *Ph. 7 095 979 98 00, Fx. 7 095 979 98 56*
Singapore *Ph. 65-2729877, Fx. 65-2734835* ***Spain*** *Ph. 34-91-661-7085, Fx. 34-91-661-9698* ***Sweden*** *Ph. 46 (0)8-55 51 27 00, Fx. 46 (0)8-55 51 27 80*
Switzerland *Ph. 01-809 55 55, Fx. 01-809 55 00* ***United Kingdom*** *Ph. 0800-181134, Fx. 01442-259118*

Sig 031799

4006192 Rev A