Mini-PROTEAN® 3
Multi-Casting Chamber

Instruction Manual

Catalog Number
165-4110

BIO-RAD

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Section 1
General Information

1.1 Introduction

The Mini-PROTEAN 3 multi-casting chamber is used to cast as many as twelve 0.5–1.5 mm thick gels simultaneously. The gels can be used in both the Mini-PROTEAN 3 cell and the Mini-PROTEAN 3 Dodeca™ Cell. After preparation, the gels can be stored up to 2 weeks at 4 ºC for future use.

1.2 Specifications

Materials of construction

Clamps	Glass filled polycarbonate
Casting chamber, sealing plate	Molded polycarbonate
Gasket	Silicone tubing
Overall size	10 cm x 10 cm x 16 cm
Weight	500 g
Compatible glass plates	Mini-PROTEAN 3 glass plates

Note: Mini-PROTEAN 3 multi-casting chamber components are not compatible with chlorinated hydrocarbons (*e.g*. chloroform), aromatic hydrocarbons (*e.g*. toluene, benzene) or acetone. Use of such solvents voids all warranties. To insure best performance of the multi-casting chamber, become fully acquainted with these instructions before use. All components should be cleaned with a suitable laboratory detergent (Bio-Rad's Cleaning Concentrate, catalog number 161-0772), rinsed thoroughly with distilled water, and dried before use.

Section 2
Loading the Chamber

1. Loosen the clamp screws, remove the sealing plate, and place the open casting chamber body face up on the benchtop. The thumbscrews should face the ceiling.
2. Start by placing a separation sheet into the chamber so that it seats at the bottom. (Be sure to remove the protective film from the separation sheet prior to use.)
3. Place a Spacer Plate (spacer side up) on top of the separation sheet.
4. Place a Short Plate on top of the Spacer Plate. Make certain that each addition is seated at the bottom of the chamber.
5. Place a separation sheet on top of the Short Plate to complete one gel sandwich.
6. Repeat steps 3–5 until you have prepared the desired number of gel sandwiches.
7. Take up the remaining space in the chamber with acrylic blocks so that the sandwiches will be held firmly in position when the sealing plate is in place. When the chamber is almost filled, Short Plates and, finally, the separation sheets can be used to fill the chamber flush to the top. If more than one glass plate is used to take up the space, insert a separation sheet between the plates to simplify separation after polymerization.

Note: To insure a good seal, the entire stack should be made as flush as possible to the top of the casting chamber, and not extend beyond it. If you overfill the chamber, monomer solution may leak out during pouring, and glass plates in the stack may break.

8. Seat the gasket firmly in the notch in the sealing plate.
9. With the clamp screws loosened, slide the sealing plate under the clamps of the casting chamber, being careful not to disturb the stack. The inlet port should match the groove at the bottom of the chamber. Gradually tighten the screws in a random fashion until tight.
10. Stand the casting chamber up, and place it on a level surface. Do not tip the chamber upside-down at this stage.

Section 3
Casting Gels

Single percentage gels can be prepared by introducing monomer from either the top or the bottom of the chamber. Gradients must be introduced from the bottom.

The first time gels of a certain thickness are cast, it is necessary to empirically determine the required volume of acrylamide. Assemble the stack as outlined in Section 2, and inject a measured volume of water through the stopcock. Prepare this volume (+5 ml) of acrylamide.

Note: Wear rubber gloves while performing the following procedure to prevent accidental exposure to unpolymerized acrylamide, which is a neurotoxin.

3.1 Casting from the Top (Non-Gradient Gels Only)

1. Attach the stopcock valve to the inlet port of the casting chamber. Make sure that the valve is in the closed position.
2. Mark a level on the chamber (with tape or a pen) at the desired separation gel length, measuring from the bottom.
3. Combine all reagents except the initiators (usually APS and TEMED), and degas the solution under vacuum for at least 15 minutes.
4. After degassing, add initiators to the gel monomer solution, and introduce the monomer into the gel sandwich closest to the sealing plate. A simple way to do this is to flow the solution down the middle of the Spacer Plate of that sandwich using a pipet or a large syringe. The groove on the bottom of the casting chamber will equilibrate the solution to each of the gel sandwiches. Monitor the filling process by observing the level of solution rising on the sandwich furthest from the sealing plate. Stop when you reach the desired gel height.
5. Overlay the gels (if a stacker is required), or insert the comb immediately.
6. Allow enough time for complete polymerization of the separating gel before removing the overlay solution (about 1 hour).
7. Prepare the stacking gel monomer solution as before and apply to the gels one at a time.
8. Insert a comb into each gel sandwich. To minimize bubble formation, insert the comb at an angle.

Note: You may also cast single percentage gels from the bottom. Use the gradient former, but just add the single percentage solution into both reservoir chambers.

3.2 Casting Gradient Gels from the Bottom

The inlet port on the Mini-PROTEAN 3 multi-casting chamber is used for casting linear and convex acrylamide gradient gels. Refer to the gradient maker instructions for preparation of gradient solutions and proper operating techniques. The Model 485 Gradient Former (catalog number 165-4120) is recommended for use with the Mini-PROTEAN 3 multi-casting chamber.

An inexperienced user should practice all steps ahead of time so that the procedure is completed quickly.

1. Place the gradient former on a magnetic stir plate and add a magnetic stir bar to the mixing chamber labeled "light". Attach the luer fitting to the stopcock valve on the inlet port. Run a piece of Tygon® tubing (1/8" ID Tygon tubing works well) from the gradient former to the luer fitting on the multi-casting chamber.
2. Determine the volume of monomer needed (see Appendix A).
3. Combine all reagents except the initiators, and degas the solution for 15 minutes.
4. Immediately prior to pouring, add TEMED and APS to both solutions, mix gently, and pour the appropriate monomer solutions into the gradient chambers. (Consult the Model 485 Gradient Former instruction manual for complete instructions.) The light solution (the one with the lower acrylamide concentration) should be placed in the mixing chamber labeled "light", and the heavy solution in the reservoir chamber labeled "heavy".
5. Turn on the stirring bar in the mixing chamber, open the tubing clamp of the gradient maker and the stopcock valve of the casting chamber, and pour the gels.

Note: If gravity flow isn't fast enough, use a peristaltic pump to pump the entire set of gradients within 10 minutes. If it is not possible to complete the operation in 10 minutes from the time initiators are added, then it might be necessary to reduce the amount of initiators (use ½ the amount of TEMED) to slow polymerization. The gradient should be poured as quickly as possible, without mixing the gradient solution in the casting chamber.

Section 4
Chamber Disassembly and Storage of Gels

Note: Wear rubber gloves while performing the following procedure to prevent accidental exposure to unpolymerized acrylamide, which is a neurotoxin.

1. Allow gel solution to polymerize for at least 1 hour.
2. Unscrew the clamp screws and remove the sealing plate carefully.
3. Remove the gels one at a time from the stack. Separate the gel sandwiches from the acrylic blocks and plates used as space-fillers.
4. Rinse off the tops of all the gels thoroughly with distilled water. Trim off excess acrylamide around the glass with a razor blade. Wash off any pieces of excess acrylamide present with distilled water.
5. Store the gels upright in a tightly sealed container or zip-lock bag. Add a few milliliters of 1X gel buffer (identical to the buffer in the gel) to the bottom of the container and to the tops of the gels to prevent them from drying out. Store tightly sealed at 4 °C. Note: If a stacking gel is required, consult Appendix B.
6. Clean the entire casting chamber thoroughly with distilled water. Residual acrylamide in the stopcock valve and inlet port can be removed using a paper clip or a syringe needle.

Section 5
Using the Gels in the Mini-PROTEAN 3 Cell or the Mini-PROTEAN 3 Dodeca Cell

1. Remove the gel(s) to be used from the storage container. Cast a stacking gel, if required, allowing 30 to 60 minutes for complete polymerization.
2. Insert gel(s) into the clamp assembly of the cell. Refer to the Mini-PROTEAN 3 cell or the Mini-PROTEAN 3 Dodeca cell manual for complete instructions.

Section 6
Product Information

Catalog Number	Description
165-4110	**Mini-PROTEAN 3 Multi-Casting Chamber** includes 8 acrylic blocks and 15 separation sheets, tapered luer fitting, and stopcock valve
165-4116	**Mini-PROTEAN 3 Multi-Casting Chamber with glass plates**, includes catalog number 161-4110 and 15 sets of 0.5 mm glass plates
165-4111	**Mini-PROTEAN 3 Multi-Casting Chamber with glass plates**, includes catalog number 161-4110 and 15 sets of 0.75 mm glass plates
165-4112	**Mini-PROTEAN 3 Multi-Casting Chamber with glass plates**, includes catalog number 161-4110 and 15 sets of 1.0 mm glass plates
165-4113	**Mini-PROTEAN 3 Multi-Casting Chamber with glass plates**, includes catalog number 161-4110 and 15 sets of 1.5 mm glass plates
165-4114	**Acrylic blocks**, 6 mm thickness, 8
165-4115	**Separation sheets**, 15
165-2913	**Replacement Cover Gaskets**, 3
165-4120	**Model 485 Gradient Former**

Section 7
Reagents for Electrophoresis

Consult the catalog or www.discover.bio-rad.com for a complete listing of Ready Gel precast gels.

Premixed Electrophoresis Buffers

161-0732 **10x Tris/Glycine/SDS Premixed Buffer**, 1 L

161-0733 **10x Tris/Boric Acid/EDTA Premixed Buffer**, 1 L

161-0734 **10x Tris/Glycine Premixed Buffer**, 1 L

161-0741 **10x TBE Extended Range Premixed Buffer**, 1 L

161-0743 **50x Tris/Acetic Acid/EDTA Premixed Buffer**, 1 L

161-0744 **10x Tris/Tricine/SDS Premixed Buffer**, 1 L

161-0772 **10x Tris/Glycine/SDS Premixed Buffer**, 5 L

161-0770 **10x Tris/Boric Acid/EDTA Premixed Buffer**, 5 L

161-0771 **10x Tris/Glycine Premixed Buffer**, 5 L

161-0758 **10x TBE Extended Range Premixed Buffer**, 6 x 1 L

161-0773 **50x Tris/Acetic Acid/EDTA Premixed Buffer**, 5 L

161-0760 **10x Tris/Tricine/SDS Premixed Buffer**, 6 x 1 L

Premixed Sample Buffers

161-0737 **Laemmli Sample Buffer**, 30 ml

161-0738 **Native Sample Buffer**, 30 ml; (Store at 2-8 °C.)

161-0739 **Tricine Sample Buffer**, 30 ml

161-0767 **Nucleic Acid Sample Buffer**, 5X, 10 ml

161-0768 **TBE-Urea Sample Buffer**, 30 ml

Acrylamide Solutions

161-0158 **30% Acrylamide/Bis Solution 37.5:1**, 500 ml

161-0140 **40% Acrylamide Solution**, 500 ml

161-0142 **2% Bis Solution Crosslinker**, 500 ml

161-0144 **40% Acrylamide/Bis Solution 19:1**, 500 ml

161-0146 **40% Acrylamide/Bis Solution 29:1**, 500 ml

161-0148 **40% Acrylamide/Bis Solution 37.5:1**, 500 ml

161-0154 **30% Acrylamide/Bis Solution 19:1**, 500 ml

161-0156 **30% Acrylamide/Bis Solution 29:1**, 500 ml

Premixed Acrylamide | Bis Powders

161-0123 **Acrylamide/Bis 19:1**, 150 g

161-0124 **Acrylamide/Bis 29:1**, 150 g

161-0125 **Acrylamide/Bis 37.5:1**, 150 g

Premixed Gel Casting Buffers

161-0798 **Resolving Gel Buffer 1.5 M Tris-HCl**, pH 8.8, 1L

161-0799 **Stacking Gel Buffer 0.5 M Tris-HCl**, pH 6.8, 1 L

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

Appendix B
Guidelines for Gel Casting

Gradient	**Non-gradient**	**Stacker**	**Overlay**	
Yes	–	No	No	Cast monomer solution to the top of the gel cassettes and insert the combs immediately.
Yes	–	Yes	Yes	Cast monomer solution to the desired resolving gel height, overlay and store in buffer once polymerized. Prior to electrophoresis cast the stacking gel and insert the combs.
–	Yes	Yes	Yes	Cast monomer solution to the desired resolving gel height, overlay and store in buffer once polymerized. Prior to electrophoresis cast the stacking gel and insert the combs.
–	Yes	Yes	Yes	Cast monomer solution to the desired resolving gel height and overlay. Once polymerized cast the stacking gel and insert the combs. Store in buffer with the combs inserted.
–	Yes	No	No	Cast monomer solution to the top of the gel cassettes and insert the combs immediately.

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