PROTEAN® plus Multi-Casting Chamber

Instruction Manual

Catalog Number
165-4160

BIO-RAD

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Section 1
General information

1.1 Introduction

The PROTEAN plus Multi-Casting Chamber allows four to twelve 1.0, 1.5 or 2.0 mm gels to be cast simultaneously for use in the PROTEAN plus Dodeca Cell. Monomer can be introduced from the top or the bottom to prepare identical linear gradient or single percentage gels. Cast gels can be stored up to 1 week at 4 ºC.

The PROTEAN plus Multi-Casting Chamber is not used to cast gels for the PROTEAN II Cell. For this application, use the PROTEAN II xi Multi-Gel Casting Chamber, catalog number 165-2025.

1.2 Specifications

Construction	
Casting chamber	fabricated acrylic
Sealing plate	fabricated acrylic with captive, spring-loaded screws
Gasket	silicone tubing
Weight	3.9 kg
Approximate size	32 cm x 23 cm x 12 cm
Gel size	20 x 20.5 (W x L) cm and 25 x 20.5 (W x L) cm in 1.0 mm, 1.5 mm, 2.0 mm thicknesses
PROTEAN plus hinged spacer plate size	26.8 x 22.5 cm (W x L)

Note: PROTEAN plus multi-casting chamber components are not compatible with chlorinated hydrocarbons (*e.g.* chloroform), aromatic hydrocarbons (*e.g.* toluene, benzene), acetone, glacial acetic acid, carbon tetrachloride, chromic acid (40%), diethyl ether, dimethyl formamide, ethyl acetate, xylene, and ethanol. 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.

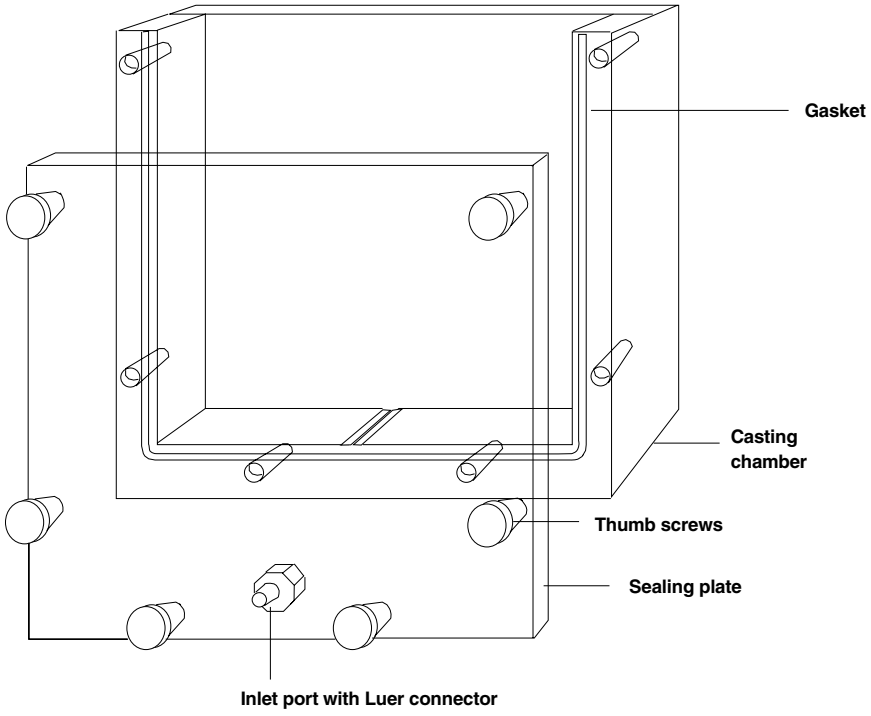

Fig. 1. PROTEAN plus Multi-Casting Chamber.

Section 2
Preparing the Multi-Casting Chamber

2.1 Loading the Chamber

1. Loosen the thumb screws, remove the sealing plate, and place the open casting chamber face up on the benchtop. The thumbscrew holes 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 the PROTEAN plus hinged spacer plate into the casting chamber body. It is easiest to insert the plate at a slight angle, hinged side first. Lower the other side into the chamber so the plate lays flat.
4. Place a separation sheet on top of the hinged spacer plate.
5. Repeat steps 3–4 until you have prepared the desired number of gel sandwiches.
6. Take up the remaining space in the multi-casting chamber with acrylic blocks and separation sheets so that the sandwiches will be held firmly in position when the sealing plate is in place.

Note: To insure a good seal, the entire stack should be made as flush as possible to the front of the multi-casting chamber, and not extend beyond it. If you overfill the multi-casting chamber, the sealing plate will not be able to create a good seal with the gasket. This causes the monomer solution to leak during pouring, and glass plates within the stack may break.

Note: Make sure that the plates and separation sheets inside the multi-casting chamber are seated at the bottom of the multi-casting chamber.

7. Seat the gasket firmly in the notch in the multi-casting chamber body.
8. With the thumb screws loosened, guide the sealing plate into place, being careful not to disturb the stack. The inlet port should match the groove at the bottom of the multi-casting chamber. Be careful not to block the inlet port with a separation sheet. Gradually tighten the screws in a random fashion until the plate is tight.
9. Stand the multi-casting chamber upright on the benchtop, and place it on a level surface. Do not tip the multi-casting chamber upside-down at this stage.

Note: It may be easier to connect the 6–8 cm piece of tubing (Section 2.2) onto the multi-casting chamber while it is still laying flat on the benchtop, prior to standing it upright.

2.2 Inlet Port Preparation

Casting gels from the TOP

Cut a piece of Tygon tubing (3 mm I.D.) about 6–8 cm in length. Connect one end of the tubing to the multi-casting chamber port by twisting the tubing side to side until it is on about 1 cm. Attach a stopcock to the other end of the tubing. Close the stopcock valve to prepare for casting (section 3).

Casting from the BOTTOM

Cut a piece of Tygon tubing (3 mm I.D.) about 6–8 cm in length (a). Connect one end of the tubing to the multi-casting chamber port by twisting the tubing side to side until it is on about 1 cm. Fit a stopcock to the other end of the tubing (b). Attach the luer connector to the stopcock (c). Tubing from the gradient former attaches to the luer connector (d). The other end of the gradient former tubing then is fitted with a luer connector (e), and this fits into a stopcock (f). This stopcock attaches directly to the gradient former. Close all valves to prepare for casting (Section 3).

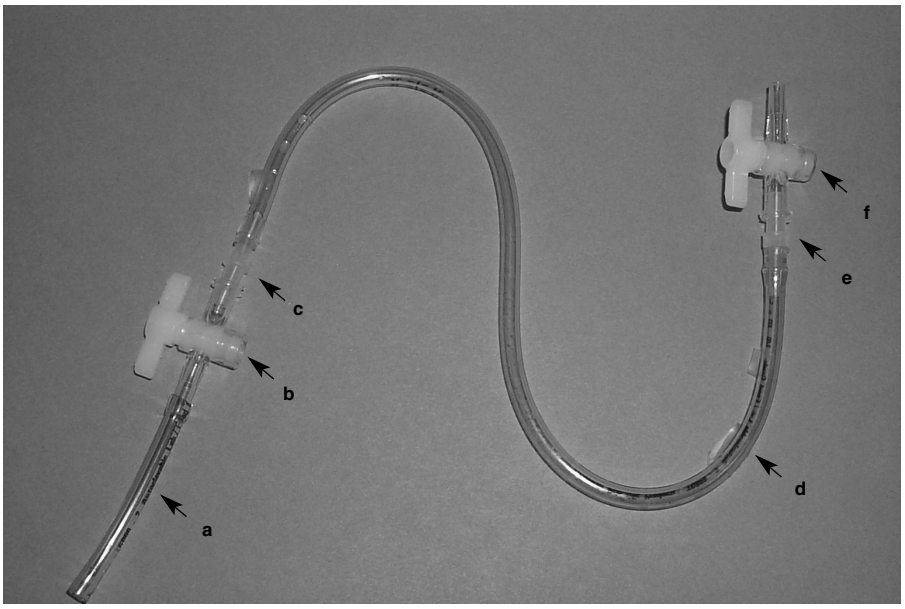

Fig. 2. Tubing and fittings to connect the Multi-Casting Chamber to the Gradient Former.

Section 3
Casting Gels

Single percentage gels (non-gradient) can be prepared by introducing monomer from either the top or bottom of the multi-casting 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 monomer solution. Assemble the stack as outlined in Section 2.1, and inject a measured volume of water through the stopcock. Prepare this volume (+ 10 ml) of monomer solution (see sample calculations in Section 3.1 and 3.2). Disassemble the multi-casting chamber and dry all components.

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

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

1. Attach a short length of tubing (8 cm of 1/8" ID Tygon® tubing is included) to the inlet port of the multi-casting chamber. Connect one of the two included stopcocks to the end of the tubing. Make sure that the valve is in the closed position.
2. Mark a level on the multi-casting chamber (with tape or a pen) at the desired separation gel length, measuring from the bottom.
3. Prepare the monomer solutions.

Note: For the larger format gels (≥ 20cm in length) it is necessary to **decrease the TEMED** amount by 50% (for a final concentration of **0.025%**). This will extend the polymerization time to approximately 20 minutes, providing time to complete casting and for the monomer solution to settle properly.

Sample Calculation: SEPARATION GEL

Objective: Cast 12 gels, 2.0mm thick, 25 x 20.5 cm, 12% acrylamide.

Place 12 clean hinged spacer plates and separation sheets in the multi-casting chamber (as described in Section 2.1) and measure the volume required to fill the plates with water. The volume required to cast the separating gel is approximately 1000 ml (*this volume will vary depending on the desired separating gel height*).

Disassemble and dry all components. Prepare 1000 ml of solution. Use the standard $C_1V_1 = C_2V_2$ equation.

To prepare 1000 ml (12%)	
(12%) (1000 ml) = (X ml) (30% acrylamide/Bis Stock)	400 ml
(0.375M Tris-Cl)(1000 ml) = (X ml) (1.5M Tris-Cl pH 8.8 Stock)	250 ml
(1000 ml) – (400 ml + 250 ml) = ml Water	350 ml
(0.05% APS) (1000 ml) = (X ml) (10% APS Stock)	5 ml
(0.025% TEMED) (1000 ml) = (X ml) (100% TEMED Stock)	250 µl

4. Combine all reagents except the initiators (usually TEMED and APS), and degas the solution under vacuum for at least 15 minutes. Degassing the solutions removes oxygen (oxygen inhibits polymerization.)

5. After degassing, add initiators to the gel monomer solution, and introduce the solution into the top of the gel sandwich closest to the sealing plate. Minimize bubble formation during casting (try to flow the solution down the middle of the sandwich using a large syringe, or direct flow down the side of the spacer with a pipette or graduated cylinder.) The groove on the bottom of the multi-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.

6. Overlay each gel **as quickly as possible** with 1.0–2.0 ml of water or water-saturated isobutanol. Optimally, the overlay solution should be applied to every gel simultaneously.

Note: each gel **must** have the same volume of overlay solution.

7. Allow enough time for complete polymerization of the separating gel before removing the overlay solution (about 1 hour). While keeping the plates assembled in the chamber, rinse the gels off thoroughly with water. After rinsing, measure the volume required to fill the stacking gel area with water. Position the gel on its side and place a rectangular piece of filter paper between the short and long plates. Dry the stacking gel area, inside the plates next to the gel, by running the filter paper from top to bottom.

Caution: do not touch or disturb the gel with the filter paper.

8. Prepare the stacking gel monomer solution (see sample calculations below) and apply to the gels one at a time.

   Note: the final concentration of TEMED is 0.1%, the concentration of Tris-Cl stock is 0.5M pH 6.8, and the final concentration is 0.125M.

   Sample Calculation: STACKING GEL

   Objective: Cast stacking gels for 12 gels, 2.0mm thick, 25 x 20.5 cm.

   Measure the volume required to fill the stacking gel area with water. The total volume required to cast all 12 stacking gels is approximately 350 ml (this volume will vary according to the height of the separating gel). Prepare 350 ml of solution. Use the standard equation, $C_1V_1 = C_2V_2$.

   To prepare 350 ml (4%)

(4%) (350 ml) = (X ml) (30% acrylamide/Bis Stock)	47 ml
(**0.125M Tris-Cl**)(350 ml) = (X ml) (**0.5M Tris-Cl pH 6.8 Stock**)	87.5 ml
(350 ml) – (47 ml + 87.5 ml) = ml Water	215.5 ml
(0.05% APS) (350 ml) = (X ml) (10% APS Stock)	1.75 ml
(***0.1% TEMED***) (350 ml) = (X ml) (100% TEMED Stock)	350 µl

9. Insert a comb into each hinged spacer plate. 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

The inlet port at the bottom of the PROTEAN plus multi-casting chamber is used for casting linear and convex acrylamide gradient gels using the Model 495 Gradient Former (catalog number 165-4121). Refer to the gradient former instruction manual for additional information and for proper operating techniques.

Note: GRADIENT GELS ONLY - the size of the V-shaped groove at the base of the multi-casting chamber has been optimized for casting twelve gels. The size of the groove was optimized so that the monomer solution enters all of the hinged spacer plates at the same time, rather than one at a time (wicking). However, the larger dimension increases the amount of monomer solution required to fill the V-groove, thus slightly altering the actual gradient formed in the gels. With this is mind, TWO solutions are recommended:

- Adjust the heavy solution by approximately 0.5% to help compensate for the V-groove. For example: If 8–16% linear gradient gels are being cast, prepare 16.5% acrylamide/Bis for the heavy solution. (see sample calculation below in Section 3.2.2)
- Adjust the amount of light and heavy solution so that they are not equal.

For example: If the total volume required to cast twelve 4–20% gradient gels is 1100 ml, prepare 555 ml of the light monomer solution (4%) and 545 ml of the heavy monomer solution (20%). (See sample calculation in Section 3.2.2 and technical directions in Section 3.2.6)

3.2.1. Place the gradient former on a magnetic stir plate and add a stir bar to the chamber labeled "light". (See Section 2.2 for inlet port preparation) Place the gradient former outlet above the top of the plate set, but minimize the length of tubing between it and the multi-casting chamber.

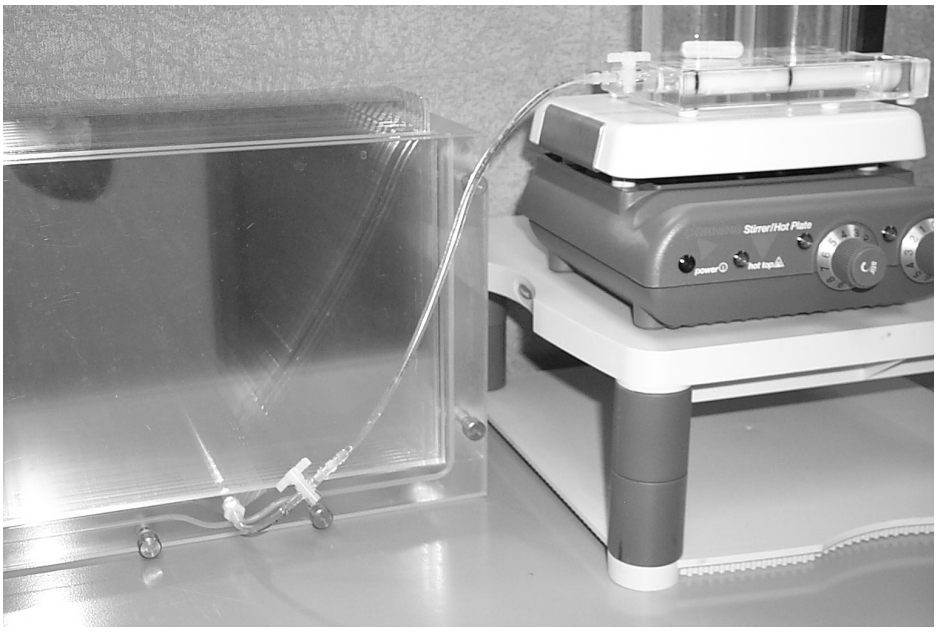

Fig. 3. Proper set-up to cast 12 gradient gels.

3.2.2. Prepare the heavy and light monomer solutions.

Note: For the larger format gels (≥ 20cm in width) it is necessary to **decrease the TEMED** concentration by 50% (for a final concentration of **0.025%**). This will extend the polymerization time to approximately 20 minutes, providing time to complete casting and for the monomer solution to settle properly. *Also, adding **10% GLYCEROL** to the HEAVY solution is important when preparing GRADIENT GELS. Glycerol adds "weight" to the heavy solution, the solution that flows into the multi-casting chamber behind the light solution. This added weight prevents the heavy solution from pushing up through the middle into the light solution. (This would cause a ripple in the gradient.) Glycerol is washed out during regular staining and protein digestion procedures which use acetonitrile and ammonium bicarbonate. It will not interfere with protein identification (using mass spectrometry).*

Sample Calculation: Linear Gradient Gels

Objective: Cast 12 gels, 2.0 mm, 25 x 20.5 cm, 8–16% acrylamide.

Place 12 clean hinged spacer plates and separation sheets in the multi-casting chamber (as described in Section 2) and measure the volume required to fill the plates with water. In this example, the volume required is 1330 ml. Disassemble and dry all components. Prepare 1340 ml of solution, 675 ml of light monomer solution (8%) and 665 ml of heavy monomer solution (16.5%). Use the standard equation, $C_1V_1 = C_2V_2$.

To prepare 675 ml (8%)	
(8%) (675 ml) = (X ml) (30% acrylamide/Bis Stock)	180 ml
(0.375M Tris-Cl)(675 ml) = (X ml) (1.5M Tris-Cl pH 8.8 Stock)	169 ml
(675 ml) – (180 ml + 169 ml) = ml Water	326 ml
(0.05% APS) (675 ml) = (X ml) (10% APS Stock)	3.375 ml
(***0.025% TEMED***) (675 ml) = (X ml) (100% TEMED Stock)	169 µl
To prepare 665 ml (16.5%)	
(16.5%) (665 ml) = (X ml) (30% acrylamide/Bis Stock)	366 ml
(0.375M Tris-Cl)(665 ml) = (X ml) (1.5M Tris-Cl pH 8.8 Stock)	166 ml
(**10% GLYCEROL**) (665 ml) = (X ml) (100% GLYCEROL)	66.5 ml
(665 ml) – (366 ml + 166 ml + 66.5 ml) = ml Water	66.5 ml
(0.05% APS) (665 ml) = (X ml) (10% APS Stock)	3.325 ml
(***0.025% TEMED***) (665 ml) = (X ml) (100% TEMED Stock)	166 µl

3.2.3. Combine all reagents except the initiators (usually APS and TEMED) and degas the solutions for 15 minutes under a vacuum. Degassing the solutions removes oxygen (oxygen inhibits polymerization.)

3.2.4. Immediately prior to pouring, add TEMED and APS to both solutions, mix gently, and pour the appropriate monomer solutions into the gradient chambers. 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". The level of the solutions in the two chambers will NOT be equal at this time.

3.2.5. Turn on the stirring bar in the mixing chamber to a steady speed and maintain this same speed throughout casting.

Note: If flow rate is kept constant, 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, the vortex that is created will also vary. The speed controls the amount of acrylamide pulled into the mixing (light) chamber from the reservoir (heavy) chamber when the connection between the two chambers is opened. Variation in stirring can disrupt the linear gradient formation. Also, the vortex should be off-center inside the mixing chamber. This can be achieved by moving the gradient former on the stir plate to offset the stir bar.

3.2.6. Start casting the gels by opening the STOPCOCK valve to the multi-casting chamber and the STOPCOCK valve to the gradient former. *The valve stem lever on the gradient former is closed at this time*. Allow the light monomer solution to enter the multi-casting chamber until the level of solution in the mixing chamber is EQUAL to the level of the heavy monomer solution in the reservoir chamber. The light solution should be close to entering the plates in the casting chamber. This should help compensate for the V-groove in the multi-casting chamber.

Note: The gradient must be poured as quickly as possible, without mixing the gradient solution in the casting chamber. In addition to decreasing the amount of initiators, a peristaltic pump can be used to pump the entire set of gradients within 10 minutes. However, if it is still not possible to complete the operation in 10 minutes with a pump, decrease the concentration of initiators to slow polymerization. An inexperienced user should practice all steps ahead of time so that the procedure of starting the gradient is completed quickly.

Note: The larger format gels (≥ 20cm in width) require a decrease in the TEMED concentration by 50% (for a final concentration of 0.025%).

3.2.7. Open the VALVE STEM lever on the gradient former once the levels are EQUAL in the two chambers, and begin mixing the solutions and creating the gradient. DO NOT allow any air bubbles to enter the plates.

3.2.8. After casting the gels to the top of the plates, CLOSE the stopcock on the multi-casting chamber and on the gradient former. Carefully remove the luer connector from the stopcock connected to the multi-casting chamber. Put the tubing into a beaker and drain the excess acrylamide from the gradient former. Insert the combs at an angle; this will help minimize bubble formation in the wells.

Caution: Immediately flush the gradient former and tubing with water to prevent polymerization of residual acrylamide within the gradient former.

Section 4
Removing Gels, Storage and Use of the Gels

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

1. Allow at least one hour for polymerization. After polymerization, remove the sealing plate from the multi-casting chamber. Remove the gels from the stack one at a time using the green gel releaser. Place the gel releaser between two plates on the hinged side. Pull the gel releaser forward, pushing against the back plate still inside the chamber. This should push and move one plate forward and release it from the rest of the stack. Lift the hinged spacer plate out of the chamber. Trim off excess acrylamide around the glass edges with the gel releaser.

Note: Polymerized acrylamide will be between the plates, and adhered to the plates, separation sheets and edges of the chamber. Use the gel releaser to remove excess acrylamide.

2. Rinse off the tops of all the gels thoroughly with distilled water. Wash off any pieces of excess acrylamide present with distilled water.

3. Store the gels upright in a tightly sealed container or zip-lock bag. Add a few milliliters of 1 X 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, it should be cast immediately prior to use.

4. Clean the entire casting chamber thoroughly with distilled water. Residual acrylamide in the stopcock valve and inlet port can be removed using a pipette tip.

Section 5
Product Information

Catalog Number	Description
165-4160	**PROTEAN plus Multi-Casting Chamber**, includes casting chamber, sealing plate, silicone gasket, tapered luer connector, leveling bubble, acrylic blocks and separation sheets. Order plates and combs separately.
165-4161	**Acrylic block (1.5 mm)**, replacement, 1
165-4162	**Acrylic block (3 mm)**, replacement, 1
165-4163	**Acrylic block (6 mm)**, replacement, 1
165-4164	**Acrylic block (12 mm)**, replacement, 1
165-4165	**Separation Sheets**, 15
165-4170	**PROTEAN plus hinged spacer plate**, 20 x 20.5 (W x L) cm, 1.0 mm, 1
165-4171	**PROTEAN plus hinged spacer plate**, 20 x 20.5 (W x L) cm, 1.5 mm, 1
165-4172	**PROTEAN plus hinged spacer plate**, 20 x 20.5 (W x L) cm, 2.0 mm, 1
165-4173	**PROTEAN plus hinged spacer plate**, 25 x 20.5 (W x L) cm, 1.0 mm, 1
165-4174	**PROTEAN plus hinged spacer plate**, 25 x 20.5 (W x L) cm, 1.5 mm, 1
165-4175	**PROTEAN plus hinged spacer plate**, 25 x 20.5 (W x L) cm, 2.0 mm, 1
165-4176	**PROTEAN plus comb**, 2-D (1 reference well), 20 cm, 1.0 mm, 1
165-4177	**PROTEAN plus comb**, 2-D (1 reference well), 20 cm, 1.5 mm, 1
165-4178	**PROTEAN plus comb**, 2-D (1 reference well), 20 cm, 2.0 mm, 1
165-4179	**PROTEAN plus comb**, 2-D (1 reference well), 25 cm, 1.0 mm, 1
165-4180	**PROTEAN plus comb**, 2-D (1 reference well), 25 cm, 1.5 mm, 1
165-4181	**PROTEAN plus comb**, 2-D (1 reference well), 25 cm, 2.0 mm, 1
165-3320	**Gel Releaser**, 5

Section 6
Reagents for Electrophoresis

Catalog Number	Description
	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

Catalog Number	Description
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 I 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

Bio-Rad Laboratories

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