Mini Prep Cell

Assembly Guide

Catalog #170-2927

BIO-RAD

Cast the Gel

1. Slide the gel tube assembly through the elution chamber top and secure it on the leveled casting stand with the three screws; hand tightening is sufficient.

2. Prepare the resolving gel solution as described in the instruction manual.

3. Use a small syringe with a PTFE (polytetrafluoroethylene) tube affixed to it to slowly fill the gel tube with monomer mixture (Figure 1). Gently tap the casting stand against the bench top to dislodge trapped air bubbles. Visually inspect the bottom of the gel for bubbles immediately after pouring the gel solution into the tube.

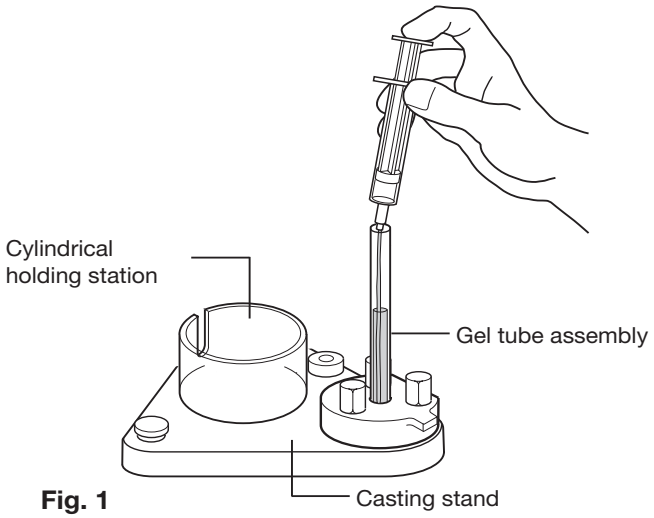

Fig. 1

4. Overlay the gel solution with water-saturated 2-butanol or tert-amyl alcohol using the narrow PTFE tube on a syringe. Allow the resolving gel to stand overnight for complete polymerization. After 1–2 hours of polymerization, replace the alcohol overlay with gel buffer.

5. Decant or aspirate the buffer overlay and, if appropriate, cast the stacking gel on top of the resolving gel. Overlay the stacking gel monomer with water-saturated 2-butanol or tert-amyl alcohol. Allow the stacking gel to polymerize for 1–2 hours.

Assemble and Purge the Elution Chamber

1. Soak the support frit, elution frit, and dialysis membrane in eletrophoresis buffer.
2. Place the molded harvest ring into the lower portion of the elution chamber then press in the gray sealing gasket around the harvest ring to hold the assembly in place.
3. Fill a syringe with electrophoresis buffer and connect it to the Luer fitting of the 1 mm PTFE tube exiting the base of the elution manifold (Figure 2). Gently push elution buffer through the elution tubing (do not pull) until buffer fills the space within the gray sealing gasket.

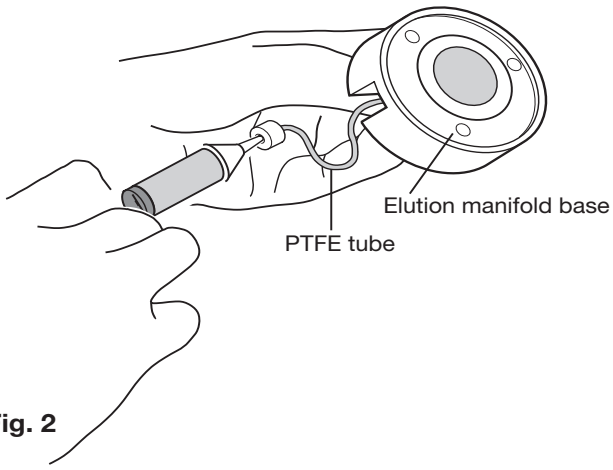

Fig. 2

4. Place the dialysis membrane on top of the support frit and then place the elution frit on top of the dialysis membrane.
5. Decant the stacking gel overlay, rinse the surface of the stacking gel with water, and loosen the three screws holding the column to the casting stand.
6. Remove the gel tube from the casting stand and place the assembly on the lower portion of the elution manifold. Hand tighten the three screws in the threaded holes in the lower portion of the manifold (Figure 3).

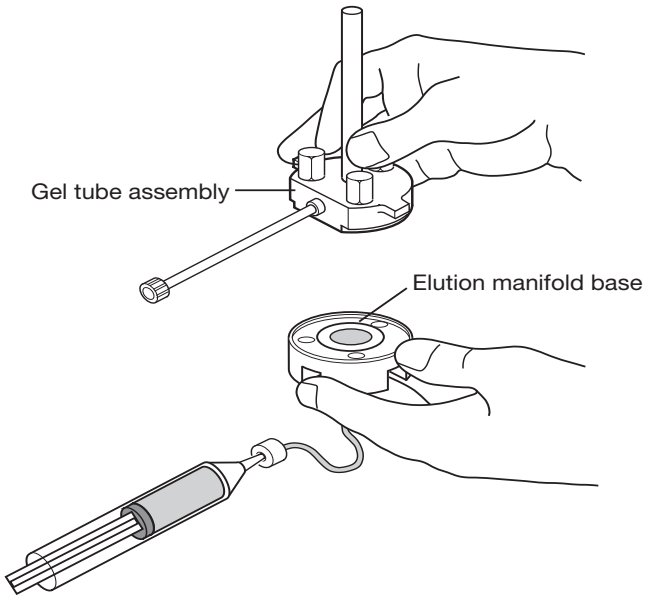

Fig. 3

7. Continue purging the assembled elution chamber until elution buffer flows out through the elution buffer feedline (Figure 4). Visually inspect the elution chamber for air bubbles. If bubbles persist, tap the elution chamber against the lab bench to free the bubbles then gently push elution buffer through the assembled elution chamber until the bubbles flow out through the elution buffer feedline.

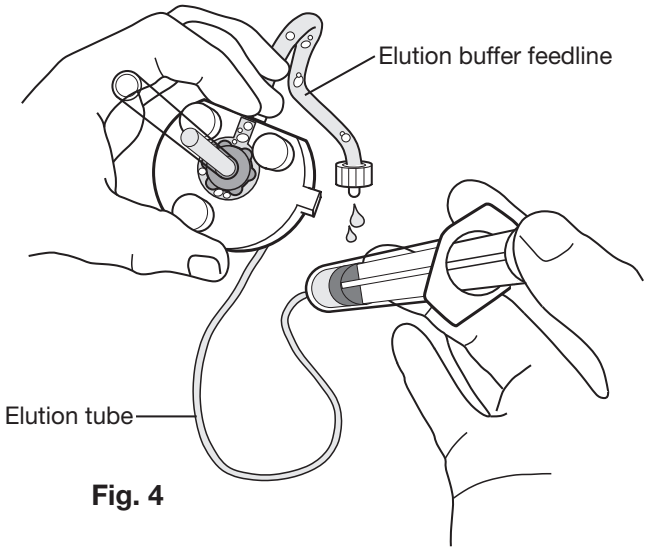

Fig. 4

Assemble the Upper and Lower Buffer Chambers

1. Place the assembled elution chamber/gel tube assembly into the cylindrical holding station on the casting stand. Allow the elution tube to pass out through the slot in the holding station.

2. Slide the top of the gel tube into the grommet in the center of the upper buffer chamber.

3. Align the two wings protruding from the elution manifold with the slots in the lower electrode housing. Twist and lock the two components in place.

4. Connect the elution buffer feed line (female Luer fitting) to the male Luer fitting attached to the elution buffer reservoir. Gently push elution buffer through the elution collection tube to prime the elution system. Priming is complete when buffer flows into the elution buffer reservoir (Figure 5).

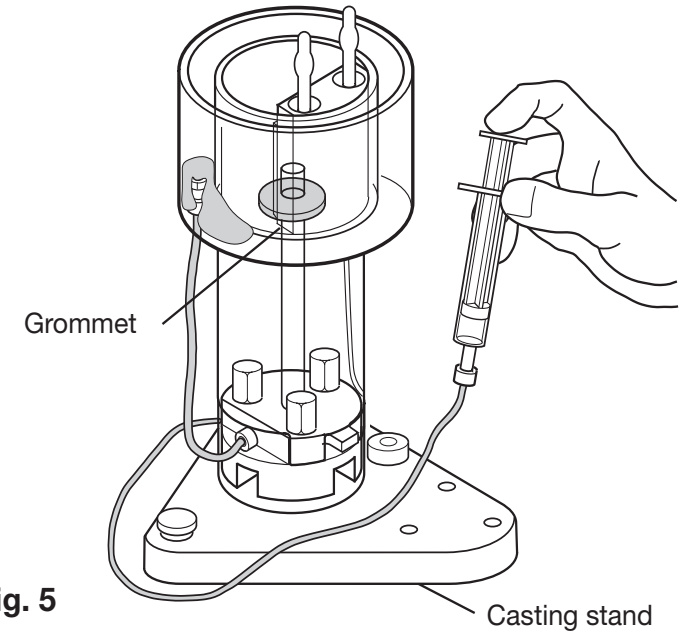

Fig. 5

5. Fill each of the upper buffer reservoirs with 100 ml of electrophoresis buffer. Add 400 ml of electrophoresis buffer to the lower buffer chamber. The level of buffer should just reach the bottom edge of the gel tube. Place the upper buffer chamber/gel tube assembly into the lower chamber.

Load the Sample

Carefully load the sample on the surface of the gel with the 1 ml syringe and attached loading tube. Layer the sample under the electrophoresis buffer (Figure 6). Make sure the gel is not punctured with the PTFE loading tube. Once the sample is loaded, place the lid on the cell and attach the cables to the power supply. Set the power supply to the appropriate settings and begin electrophoresis.

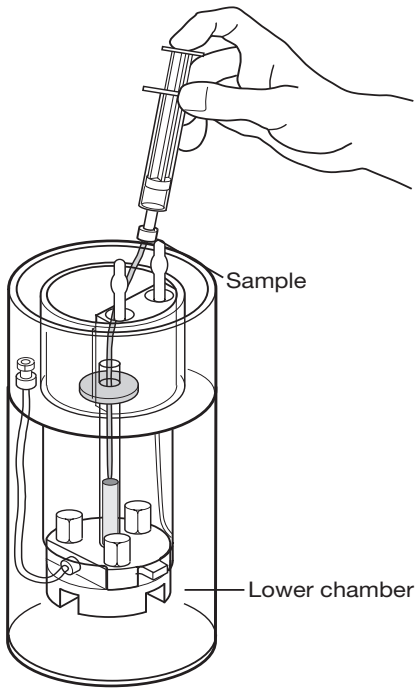

Fig. 6

Set the Elution Rate

The recommended elution buffer flow rate is 75–100 µl/min. Fractions collected for 2.5 minutes (~200–250 µl) usually maintain sufficient separation of eluted proteins. Fraction collection should begin after the ion/dye front has eluted.

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