Using EDVOTEK's Polyacrylamide Gels

EDVOTEK's Precast Polyacrylamide Gels

ABOUT THIS PRODUCT:

- 4-20% Tris-Glycine-SDS Precast Polyacrylamide Gel (PAGE)
- Size: 9 x 10 cm
- Designed for Separating Protein Fragments 20-100 kDa
- Each Gel Has 10 Wells
- Well Volume is 30 µL
- Requires Refrigeration. Use within 3 months of receipt.





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Cat. #650 One precast gel.

Cat. #651 Three precast gels.

Cat. #652 Six precast gels.

NOTE:

EDVOTEK® Cat. #638, Fine Tip Micropipette Tips are recommended for loading samples into polyacrylamide gels. A regular micropipette tip may damage the cassette and result in the loss of protein samples.

EDVOTEK®

1.800.EDVOTEK www.edvotek.com

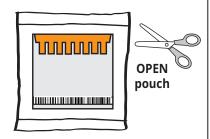
Preparing PAGE Gel and Chamber

NOTE: Although precast polyacrylamide gels and protein chambers will vary slightly in design, the procedure for their use will be similar.



Wear gloves and safety goggles.

1.



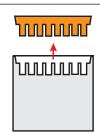
OPEN the pouch containing the gel cassette. Remove the cassette and place on bench with the shorter front plate facing up.

2. REMOVE tape (if present)



Gels may feature a sticker or tape at the bottom of the front plate. **REMOVE** the tape (if present) to expose the bottom of the gel.

REMOVE comb



Carefully **REMOVE** the comb by gently pulling upwards. Pull the comb straight up to prevent damage to the wells of the gel.

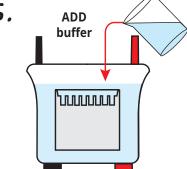
4. **INSERT** gel (short plate inward)



INSERT the gel into the electrophoresis chamber. Orient the gel according to the manufacturer's instructions.

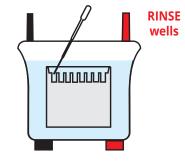
NOTE: For EDVOTEK® vertical electrophoresis chambers, the short plate should face the interior.

5.



ADD diluted electrophoresis buffer to the chamber. The buffer should cover the top of the front, shorter plate.

6.



RINSE each well by squirting electrophoresis buffer into the wells using a transfer pipet.

The gel is now ready for sample loading.

Loading the Protein Samples

NOTE: EDVOTEK® Cat. #638, Fine Tip Micropipette Tips are recommended for loading samples into polyacrylamide gels. A regular micropipette tip may damage the cassette and result in the loss of protein samples.

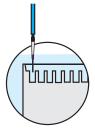


Wear gloves and safety goggles.

1.



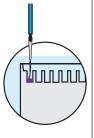
Using a fresh fine tip micropipette tip, **MEASURE** between 5 - 25 µL of your protein sample. 2.



PLACE the pipette tip under the buffer and directly above the sample well, resting gently against the back plate of the gel cassette. 3.

Slowly

DISPENSE the sample by depressing the plunger.



4. REPEAT these steps for remaining samples. *NOTE: Be sure to change pipette tips between loading each sample!*



Once all samples have been loaded, carefully **PLACE** the cover onto the electrode terminals.

6. CONNECT the electrical leads to the power supply.

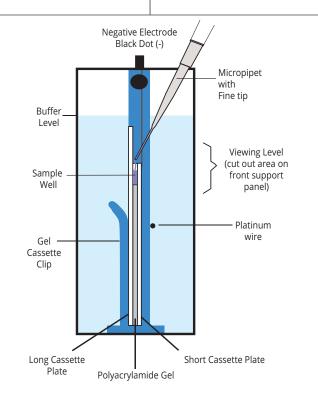


7. SET the voltage of the power supply and PERFORM electrophoresis (See Table A for time and voltage guidelines). Allow the proteins to separate on the gel for the recommended length of time, or until the tracking dye reaches near the bottom of the gel.

Table A	Time & Voltage Guidelines	
	Recommended Time	
Volts	Minimum	Optimal
100	70 min.	90 min.
125	50 min.	60 min.
150	40 min.	50 min.

8. After the electrophoresis is finished, **TURN OFF** the power supply, disconnect the leads, and carefully **REMOVE** the cover. The gel can now be removed from the chamber. Immediately proceed to staining.





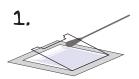
A polyacrylamide gel cassette in the EDVOTEK® MV10 Vertical Electrophoresis Apparatus (Cat. #581).

Staining the Gel

NOTE: Gloves must be worn during this procedure. Avoid touching the gel or Protein InstaStain® paper without gloves. Polyacrylamide gels are very thin and fragile. Use care in handling to avoid tearing the gel.



Wear gloves and safety goggles.

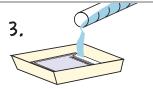


After electrophoresis, LAY the cassette down and REMOVE the front plate by placing a thin spatula or screwdriver at the side edge and gently lift it away from the larger back plate. In most cases, the gel will stay on the back plate. If it partially pulls away with the front plate, let it fall onto the back plate. Handle very carefully as the thin gels are extremely fragile.

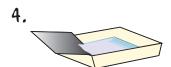
2.



TRANSFER the gel on the back plate to a clean tray.



ADD a sufficient volume (approximately 100 mL) of the staining/destaining solution into the tray to **COVER** the gel and back plate. (Use enough solution to cover the gel.)



Carefully **REMOVE** the back plate from the tray, leaving just the gel in the tray containing the fixative solution.

NOTE: If the gel sticks to the plate, pipette some of staining/ destaining solution onto the gel and gently nudge the gel off the plate.



Gently FLOAT a sheet of Protein InstaStain® with the stain side (blue side down) in the staining/destaining solution. **COVER** the gel with plastic wrap to prevent evaporation.

6.



Allow the Protein InstaStain® paper to **STAIN** the gel for about an hour at room temperature with gentle occasional or continuous agitation.





AGITATE on a rocking platform or just on the lab bench for 2-3 hours. Gels may also be stored overnight if desired.

NOTE: Overnight staining yields a more optimal result. Pour off the staining solution from step 7 the following day and add fresh staining/destaining solution to cover the gel.

8.



After staining, Protein bands will appear medium to dark blue against a light background* and will be ready for excellent photographic results.

*If the gel is too dark, destain at room temperature with continuous agitation in several changes of fresh staining/destaining solution until the appearance and contrast of the protein bands against the background improve.