Mini-PROTEAN® Empty Cassette Gel Casting Instructions

Instructions

1. Prepare the resolving gel acrylamide solution by combining all reagents except ammonium persulfate (APS) and TEMED. Degas the solution under a vacuum for at least 15 min.
2. The Mini-PROTEAN cassette should be held vertically for gel casting. The AnyGel™ stand (catalog #165-4131) or a test tube rack works well to stabilize the cassette during casting.
3. Add APS and TEMED to the degassed acrylamide solution and pour the solution into the cassette. Pour the solution smoothly to prevent it from mixing with air. Fill the cassette until the solution is 1 cm below the teeth of the comb.
4. Immediately overlay the acrylamide solution with distilled water. Apply the overlay slowly, with a steady, even rate of delivery to prevent mixing. DO NOT use alcohol overlay solutions, as these may discolor the cassette.
5. Allow the gel to polymerize for 45 min to 1 hr. Rinse off the overlay solution with distilled water.
6. Prepare the stacking gel acrylamide solution. Combine all reagents except APS and TEMED and degas under vacuum for at least 15 min.
7. Dry the area above the separating gel with filter paper before pouring the stacking gel.
8. Place a comb in the cassette and tilt it so that the teeth are at a slight (~10º) angle. This will prevent air from being trapped under the comb teeth while the acrylamide solution is being poured.
9. Add APS and TEMED to the degassed acrylamide solution and pour the solution down the edge of the cassette nearest to the upturned side of the comb. Pour until all the teeth have been covered by solution. Then properly align the comb in the cassette and add solution to fill completely.
10. Allow the gel to polymerize 30–45 min.

Stock Solutions

30% Acrylamide/bis (37.5:1)

29.2 g acrylamide
0.8 g N,N'-bis-methylene-acrylamide

Make to 100 ml with deionized water. Filter and store at 4ºC in the dark.

Or, use

30% Acrylamide/Bis Solution, 37.5:1, 500 ml (catalog #161-0158)
Preweighed Acrylamide/Bis Powder, 37.5:1, 30 g (catalog #161-0122)

1.5 M Tris-HCl, pH 8.8

18.15 g Tris base
~60 ml deionized water

Adjust to pH 8.8 with 6 N HCl.
Make to 100 ml with deionized water and store at 4ºC in the dark.

Or, use

1.5 M Tris-HCl, pH 8.8, 1 L (catalog #161-0798)

0.5 M Tris-HCl, pH 6.8

6 g Tris base
~60 ml deionized water

Adjust to pH 6.8 with 6 N HCl.
Make to 100 ml with deionized water and store at 4ºC in the dark.

Or, use

0.5 M Tris-HCl, pH 6.8, 1 L (catalog #161-0799)

10% (w/v) SDS

Dissolve 10 g SDS in 90 ml water and bring to 100 ml with deionized water.

Or, use

SDS Solution, 10% (w/v), 250 ml (catalog #161-0416)

	Stacking Gel	Resolving Gel		
	4%	7.5%	12%	X%
30% Acrylamide/bis	1.32 ml	2.5 ml	4.0 ml	0.33•X ml
0.5 M Tris-HCl, pH 6.8	2.52 ml	—	—	—
1.5 M Tris-HCl, pH 8.8	—	2.5 ml	2.5 mnl	2.5 ml
10% SDS	100 µl	100 µl	100 µl	100 µl
Distilled deionized water	6 ml	4.85 ml	3.35 ml	7.35 – (0.33•X) ml
TEMED	10 µl	5 µl	5 µl	5 µl
10% APS	50 µl	50 µl	50 µl	50 µl
Total Volume	10 ml	10 ml	10 ml	10 ml

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