

Criterion® Cassette Hand 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 Criterion cassette should be held vertically for gel casting. The Single-row AnyGel casting stand (catalog #165-4131) can be used to stabilize the cassette during casting.
3. Add APS and TEMED to the degassed acrylamide solution and pour the solution into the Criterion cassette. Pour the solution smoothly to prevent it from mixing with air. Fill the Criterion cassette to the top of the label tape, or 1 cm below the teeth of the comb.
4. Immediately overlay the acrylamide solution with 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 the Criterion comb in the Criterion cassette and tilt it so that the teeth are at a slight (~10°) 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 Criterion 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 Criterion cassette and add solution to fill completely.
10. Allow the gel to polymerize for 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 4°C 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 4°C 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 4°C 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	2.0 ml	3.75 ml	6.0 ml	0.5 · X ml
0.5 M Tris-HCl, pH 6.8	3.75 ml	–	–	–
1.5 M Tris-HCl, pH 8.8	–	3.75 ml	3.75 ml	3.75 ml
Distilled deionized water	9.0 ml	7.3 ml	5.0 ml	11.0 - (0.5 · X) ml
10% SDS	150 µl	150 µl	150 µl	150 µl
TEMED	15 µl	7.5 µl	7.5 µl	7.5 µl
10% APS	75 µl	75 µl	75 µl	75 µl
Total Volume	15 ml	15 ml	15 ml	15 ml