30% acrylamide-methylenebisacrylamide solution (29:1)

Project number: A665714

Storage conditions: 2-8° C.

Products

individual parts making up a compound	500ml
30% Acr-Bis (29:1)	2 x 250ml

Products

30% Acrylamide:Bis Solution (29:1) is an aqueous solution of 30% acrylamide, N,N′-methylenebisacrylamide (29:1), referred to as 30% Acr-Bis (29:1). This product is commonly used to prepare denaturing and non-denaturing polyacrylamide (PAGE) gels of various concentrations for routine protein or nucleic acid electrophoresis experiments, and is easy to use.

procedure

According to the molecular weight size of the target protein, select the appropriate concentration of PAGE separation gel preparation, the optimal gel concentration, please refer to Exhibit 1.

I Infusion of separating gel (please refer to Exhibit 2 for the amount of each reagent used)

1. Refer to the gel mold instructions and assemble the gel mold.

Note: The addition of the upper sieve plate helps to maintain uniform contact between the filler and the sample when adding samples, and the addition of the upper sieve plate can be selected according to the actual situation.

2. Mix different volumes of 30% Acr-Bis (29:1), separating gel buffer and pure water in a small beaker or test tube.

3. Add 10% APS and TEMED, stirring gently to mix well and to avoid air bubbles.

4. Fill the gel mold with the appropriate amount of separator gel solution (for mini-gel, the gel solution is added approximately to the top of the front glass plate).

(1.5 cm or about 0.5 cm from the comb teeth is sufficient), and then gently cover the separating gel solution with a 1 cm layer of water to keep the gel surface flat.

5. Allow to stand for 30-60 minutes, after a clear interface between the separating gel and the water layer has appeared and the surface gel has polymerized.

II Filling of concentrated gel (please refer to Exhibit 3 for the amount of each reagent used)

1. Remove the water layer covering the separation gel.

2. Mix different volumes of 30% Acr-Bis (29:1), Gum Concentrate Buffer and pure water in a small beaker or test tube.

3. Add 10% ammonium persulfate and TEMED, stirring gently to mix well and to avoid air bubbles.
4. Add the concentrated gel solution to the top of the separation gel until the gel solution reaches the top of the front glass plate.
5. Insert the comb into the gel to avoid air bubbles.
6. Allow to stand for 10 to 20 minutes and wait for the gel concentrate to polymerize.
7. After the gel has polymerized, carefully remove the comb so as not to damage the spiking hole.
8. Perform routine electrophoresis operations.

schedules

Exhibit 1. Concentration and optimal separation range of SDS-PAGE separation gel

SDS-PAGE Separation gel concentration	Optimum separation range
6% Gum	50-150 kD
8% Gum	30-90 kD
10% Gum	20-80 kD
12% Gum	12-60 kD
15% Gum	10-40 kD

Schedule 2. Preparation of SDS-PAGE Separation Gel

Separation gel concentration	Gel volume	Volume of each component required (in ml)				
		purified water	30% Acr-Bis (29:1)	SDS-PAGE Separating Gel Buffer (4 x)	10%A PS	TEMED
6%	5 ml	2.75	1.0	1.25	0.05	0.004
	10 ml	5.5	2.0	2.5	0.1	0.008
	15 ml	8.25	3.0	3.75	0.15	0.012
	20 ml	11	4.0	5	0.2	0.016
8%	5 ml	2.42	1.33	1.25	0.05	0.003
	10 ml	4.8	2.7	2.5	0.1	0.006
	15 ml	7.25	4.0	3.75	0.15	0.009
	20 ml	9.7	5.3	5	0.2	0.012
10%	5 ml	2.08	1.67	1.25	0.05	0.002
	10 ml	4.17	3.33	2.5	0.1	0.004
	15 ml	6.25	5.0	3.75	0.15	0.006
	20 ml	8.3	6.7	5	0.2	0.008
12%	5 ml	1.75	2.0	1.25	0.05	0.002
	10 ml	3.5	4.0	2.5	0.1	0.004
	15ml	5.25	6.0	3.75	0.15	0.006
	20ml	7.0	8.0	5	0.2	0.008

15%	5ml	1.25	2.5	1.25	0.05	0.002
	10ml	2.5	5.0	2.5	0.1	0.004
	15ml	3.75	7.5	3.75	0.15	0.006
	20ml	5	10.0	5	0.2	0.008

Schedule 3. Preparation of 5% SDS-PAGE gel concentrate

Gel volume	Volume of each component required (in ml)				
	purified water	30% Acr-Bis (29:1)	SDS-PAGE Stacking Gel Buffer (4×)	10% AP S	TEMED
2ml	1.14	0.34	0.5	0.02	0.002
4ml	2.28	0.68	1	0.04	0.004
6ml	3.42	1.02	1.5	0.06	0.006
8ml	4.56	1.36	2.0	0.08	0.008