SDS-PAGE separation gel buffer (4×)

Project number: S670007

Storage conditions: 2-8° C.

Products

This product is a buffer for the preparation of SDS-PAGE separation gels, which can be used to prepare denaturing and non-denaturing PAGE gels of various concentrations, convenient and quick. 10% SDS has been added to the product, so there is no need to add it separately.

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 Schedule 2 for the amount of each reagent used)

1. Refer to the gel mold instructions and assemble the gel mold.
2. Mix different volumes of 30% Acr-Bis (29:1), SDS-PAGE Separating Gel Buffer (4×) Separating Gel Buffer and pure water in a small beaker or test tube.
3. Add 10% APS and TEMED, stir gently to mix well and avoid air bubbles.
4. In the gel mold filled with the appropriate amount of separation gel solution (for mini-gel, the gel solution added to about 1.5cm from the top of the front glass plate or about 0.5cm from the teeth of the comb can be), and then gently covered with a layer of 1cm of water on the separation gel solution, so that the surface of the gel to maintain a flat.
5. Let it stand for 30-60 minutes, after a clear interface appears between the separated gel and the water layer, the surface gel has been polymerized.

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

1. Remove the water layer covering the separator gel.
2. Mix different volumes of 30% Acr-Bis (29:1), concentrated gel buffer and pure water in a small beaker or test tube.
3. Add 10% ammonium persulfate and TEMED, stir gently to mix well and 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. Let it stand for 10~20 minutes and wait for the concentrated gel to polymerize.
7. After the gel has polymerized, carefully pull out the comb so as not to damage the spiking hole.
8. Perform routine electrophoresis operations.

Schedules

Exhibit 1. Concentration of SDS-PAGE Separation Gel and Optimal Separation Range

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)	Separation gel buffer (4×)	10% APS	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
	15 ml	5.25	6.0	3.75	0.15	0.006
	20 ml	7.0	8.0	5	0.2	0.008
15%	5 ml	1.25	2.5	1.25	0.05	0.002
	10 ml	2.5	5.0	2.5	0.1	0.004
	15 ml	3.75	7.5	3.75	0.15	0.006
	20 ml	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)	Concentrated gel buffer (4×)	10% APS	TEMED
2 ml	1.14	0.34	0.5	0.02	0.002

4 ml	2.28	0.68	1	0.04	0.004
6 ml	3.42	1.02	1.5	0.06	0.006
8 ml	4.56	1.36	2.0	0.08	0.008