

### SDS-PAGE Gel Kit

Project number: S665689

Storage conditions: 2-8° C.

#### Products

individual parts making up a compound	40-60 gels	200-300 gels
30% Acr-Bis (29:1)	100m1	2 x 250m1
SDS-PAGE Separating Gel Buffer $(4\times)$	100ml	500m1
SDS-PAGE Stacking Gel Buffer (4×)	50m1	250m1
APS	0.5g	2.5g
TEMED	1ml	5m1

The APS (Ammonium Persulfate) supplied with this product is a solid powder, which is dissolved in purified water before use to form a 10% APS solution (0.5 g APS plus 5 ml of purified water, 2.5 g APS plus 25 ml of purified water), and the solution is dispensed and placed at -20° C for storage, which is usually effective for six months. The solution can be placed in use and stored at 4° C for two weeks.

#### Products

This product includes a full set of reagents required for the preparation of SDS-PAGE gels, only need to prepare their own pure water, you can prepare high quality denaturing PAGE gels of various concentrations, convenient and fast. 10% SDS has been added to the separation buffer and concentration buffer, so there is no need to add SDS to the buffer.

#### Caveat

- 1.10% APS is prepared and stored at -20 degrees C. APS solution is unstable and should be stored at room temperature for as little time as possible, and returned to the refrigerator immediately after each use to prevent failure; if the gel polymerization time is found to be prolonged, consideration should be given to replacing it.
- -10% APS kept at 20 degrees.
- 2. The cohesion speed of PAGE gel is closely related to the temperature and the dosage of APS and TEMED; in the case of other conditions remain unchanged, the polymerization speed of PAGE gel can be controlled by changing the dosage of APS and TEMED, and the gel polymerization is too fast for operation; the amount of APS



and TEMED in the attached table can be used for reference, and should be adjusted appropriately according to the actual operation.

- 3. During the gel preparation process, especially the liquid mixing step, the generation of air bubbles should be avoided as much as possible.
- 4. Be careful when adding pure water to the upper layer of the separation gel, and do not be too fast when adding water.
- 5. Acrylamide is neurotoxic, please wear lab coat and disposable gloves when handling.
- 6. This product is for scientific research only and cannot be used for human experimentation or human treatment.

#### 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 3 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), SDS-PAGE 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. Fill the gel mold with the appropriate amount of separator gel solution (for mini-gel, add gel solution to about 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. 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 of concentrated gel (please refer to Exhibit 2 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), SDS-PAGE Stacking 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. 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

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

SDS-PAGE 分离胶浓度	最佳分离范围	
6%胶	50-150 kD	
8%胶	30-90 kD	
10%胶	20-80 kD	
12%胶	12-60 kD	
15%胶	10-40 kD	

Schedule 2. Preparation of 5% SDS-PAGE gel concentrate

凝胶 _	所需各组分体积(单位: ml)					
	纯水	30%Acr-Bis(29:1)	SDS-PAGE Stacking Gel Buffer (4×)	10%APS	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	

Schedule 3. Preparation of SDS-PAGE Separation  $\ensuremath{\operatorname{Gel}}$ 

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分离胶 浓度	凝胶体积	所需各组分体积(单位: ml)					
		纯水	30%Acr-Bis(29:1)	SDS-PAGE Separating Gel Buffer (4×)	10%APS	TEMED	
6%	5ml	2.75	1.0	1.25	0.05	0.004	
	10ml	5.5	2.0	2.5	0.1	0.008	
	15ml	8.25	3.0	3.75	0.15	0.012	
	20ml	11	4.0	5	0.2	0.016	
8%	5ml	2.42	1.33	1.25	0.05	0.003	
	10ml	4.8	2.7	2.5	0.1	0.006	
	15ml	7.25	4.0	3.75	0.15	0.009	
	20ml	9.7	5.3	5	0.2	0.012	
10%	5ml	2.08	1.67	1.25	0.05	0.002	
	10ml	4.17	3.33	2.5	0.1	0.004	
	15ml	6.25	5.0	3.75	0.15	0.006	
	20ml	8.3	6.7	5	0.2	0.008	
12%	5ml	1.75	2.0	1.25	0.05	0.002	
	10ml	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	