TECHNICAL MANUAL

Do-It-Yourself SDS-PAGE Gel kit

- **SKU CODE:** AGEL3522
- **SIZE:** 10 Assays
- **RUO:** Research-Use-Only

Revised: 02/26

Do-It-Yourself SDS-PAGE Gel kit

Please read entire manual carefully before starting experiment.

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1. Product Description

Assay Genie's is Do-It-Yourself SDS-PAGE Gel Kit is a classic SDS-PAGE gel preparation system containing all reagents required for casting polyacrylamide gels. Users only need standard electrophoresis apparatus and ddH_2O to complete gel preparation. The Gel Mix components in this kit combine essential buffer ingredients (such as SDS and Tris-HCl) to streamline and simplify the gel-casting process. The Separating Gel Mix contains Tris-HCl (pH 8.8) and SDS, while the Stacking Gel Mix contains Tris-HCl (pH 6.8) and SDS.

2. Storage & Expiry

The components of this product should be stored separately for up to 6 months. For detailed storage instructions of individual kit components, please refer to Section 3. The expiration date is indicated on the outer label of the kit box.

- 30% Acr-Bis (29:1) and TEMED should be stored at 2~8°C in dark.
- 10% APS should be split into small tubes and stored at -20°C for 6 months. If store at 2~8°C, please do not use it after one week.
- Other reagents should be stored at RT.

3. Kit Contents

No	Component Name	10 Assays	Storage
1	30% Acr-Bis (29:1)	45 mL	2~8°C
2	Separating Gel Mix	22 mL	RT
3	Stacking Gel Mix	6 mL	RT
4	APS	170 mg	RT
5	TEMED	100 µL	2~8°C

4. Cautions

1. For optimal assay performance, use this reagent within 6 months. Avoid repeated freeze–thaw cycles.
2. TEMED is volatile; ensure the cap is tightly closed after use. Gel polymerization rates vary with temperature, so the amount of TEMED may be adjusted accordingly to achieve the desired gelation speed.
3. Avoid introducing bubbles, as they may interfere with experimental results.
4. Gently overlay the separating gel with ddH_2O or absolute ethanol to ensure a smooth, even interface.
5. For optimal assay performance, use this reagent within 6 months. Avoid repeated freeze–thaw cycles.
6. TEMED is volatile; ensure the cap is tightly closed after use. Gel polymerization rates vary with temperature, so the amount of TEMED may be adjusted accordingly to achieve the desired gelation speed.
7. Avoid introducing bubbles, as they may interfere with experimental results.
8. Gently overlay the separating gel with ddH_2O or absolute ethanol to ensure a smooth, even interface.
9. Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our reagents, we are not responsible for any loss of samples during use. We advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.

5. Instructions

According to the different molecular weight of the target protein, different concentration of separating gel should be prepared.

1. Comparison of protein separation linear range is shown in Schedule 1.

2. Sample volume of each component corresponding to different stacking gel volumes is shown in Schedule 2.

3. Sample volume of each component corresponding to different separation gel volumes is shown in Schedule 3.

6. Procedure

1. Preparation of 10% APS: add distilled water or DI water (add 10μL of water to 1mg APS) to APS to prepare a 10% APS, For example: add 1.7mL distilled water to170mg APS, the mixture is 10% APS. 10% APS should be split into small tubes and stored at -20°C.
2. Choose the clean gel mold and complete the assembly.
3. Refer to Schedule 3, prepare the separating gel of the required concentration and volume. Add appropriate amount of ddH2O, 30% Acr-Bis (29:1) and Separating Gel Mix to a clean beaker, mix it.
4. Add appropriate amount of 10% APS and TEMED, mix gently and avoid bubbles.
5. Add the mixture gel to the assembled gel mold immediately. Then add 1~2 mL ddH2O or absolute alcohol on the separating gel to flatten the separating gel level.
6. Keep at RT for 30~60 min until the separating gel solidified completely.
7. Pour out the ddH2O or absolute ethanol, absorb the residual liquid with absorbent paper.
8. Refer to Schedule 2, prepare the stacking gel of the required volume. Add appropriate amount of ddH2O,30% Acr-Bis (29:1) and stacking Gel Mix to a clean beaker, mix it.
9. Add appropriate amount of 10% APS and TEMED, mix gently and avoid bubbles.

10. Add the mixture gel upon to the separating gel immediately. Insert comb teeth carefully, avoid bubbles.
11. Keep at RT for 30~40 min until the stacking gel solidified completely.
12. Pull out the comb teeth carefully. Follow the SDS-PAGE experiment.

Schedule 1. Comparison of protein separation linear range

Concentration of separating gel	**Linear separation range**
6%	50~150kDa
8%	30~90kDa
10%	20~80kDa
12%	12~60kDa
15%	10~40kDa

Schedule 2. Preparation of stacking gel

Components	**Sample volume of each component corresponding to different stacking gel volumes**							
	1 mL	2mL	3mL	4mL	5mL	6mL	8mL	10mL
5% Stacking Gel								
H_2O	0.68	1.4	2.1	2.7	3.4	4.1	5.5	6.8
30% Acr-Bis	0.17	0.33	0.5	0.67	0.83	1.0	1.3	1.7
Stacking Gel Mix	0.14	0.27	0.41	0.54	0.68	0.81	1.08	1.35
10% APS	0.01	0.02	0.03	0.04	0.05	0.06	0.08	0.1
TEMED	0.001	0.002	0.003	0.004	0.005	0.006	0.008	0.01

Schedule 3. Preparation of separating gel

Components	**Sample volume of each component corresponding to different separation gel volume**							
	5mL	10mL	15mL	20mL	25mL	30mL	40mL	50mL
6% Gel								
ddH_2O	2.6	5.3	7.9	10.6	13.2	15.9	21.2	26.5
30% Acr-Bis	1.0	2.0	3.0	4.0	5.0	6.0	8.0	10.0
Separating Gel Mix	1.35	2.6	3.95	5.2	6.55	7.8	10.4	13.0
10% APS	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED	0.004	0.008	0.012	0.016	0.02	0.024	0.032	0.04
8% Gel								
ddH_2O	2.3	4.6	6.9	9.3	11.5	13.9	18.5	23.2
30% Acr-Bis	1.3	2.5	4.0	5.3	6.7	8.0	10.7	13.3
Separating Gel Mix	1.35	2.6	3.95	5.2	6.55	7.8	10.4	13.0
10% APS	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED	0.003	0.006	0.009	0.012	0.015	0.018	0.024	0.03
10% Gel								
ddH_2O	1.9	4.0	5.9	7.9	9.9	11.9	15.9	19.8
30% Acr-Bis	1.7	3.3	5.0	6.7	8.3	10.0	13.3	16.7
Separating Gel Mix	1.35	2.6	3.95	5.2	6.55	7.8	10.4	13.0
10% APS	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02
12% Gel								
ddH_2O	1.6	3.3	4.9	6.6	8.2	9.9	15.9	16.5
30% Acr-Bis	2.0	4.0	6.0	8.0	10.0	12.0	13.3	20.0
Separating Gel Mix	1.35	2.6	3.95	5.2	6.55	7.8	10.4	13.0
10% APS	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02
15% Gel								
ddH_2O	1.1	2.3	3.4	4.6	5.7	6.9	9.2	11.5
30% Acr-Bis	2.5	5.0	7.5	10.0	12.5	15.0	20.0	25.0
Separating Gel Mix	1.35	2.6	3.95	5.2	6.55	7.8	10.4	13.0
10% APS	0.05	0.1	0.15	0.2	0.25	0.3	0.3	0.5
TEMED	0.002	0.004	0.006	0.006	0.01	0.01	0.016	0.02

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.