One-Step PAGE Gel Fast Preparation Kit (12.5%)

Cat.:BR4C334

V01

Product Description

One-Step PAGE Gel Fast Preparation Kit (12.5%) is suitable for Tris-glycine electrophoresis systems and uses premixed formulations for stacking gel and separating gel. Gel casting can be completed simply by adding Improved Coagulant, making the workflow convenient and fast. The prepared stacking gel is red, allowing the sample wells to be clearly distinguished for convenient loading. The red stacking gel can also be used to differentiate gels that contain different samples. This product is supplied with Improved Coagulant, which provides improved stability and catalytic efficiency, so no additional TEMED is required during gel preparation.

Components

Components	BR4C334-01 125 gels (0.75 mm)
Stacking Gel Solution (2×)	80 mL
Red Stacking Gel Buffer (2×)	80 mL
Separating Gel Solution 12.5% (2×)	250 mL
Separating Gel Buffer 12.5% (2×)	250 mL
Improved Coagulant	8 mL

Storage

Store Improved Coagulant at -20±5°C. Store all other reagents at 2-8°C.

Notes

1. For Research Use Only. Not for use in diagnostic procedures.
2. The amount of Improved Coagulant is for reference only. The actual amount may be adjusted according to individual experimental habits and experience. Using more coagulant accelerates gelation, and vice versa. The gelation rate of PAGE gels is closely related to temperature and the amount of coagulant used.
3. Do not mix components from kits with different concentrations; otherwise, gel casting and electrophoresis performance may be affected.
4. The gelation rate is strongly positively correlated with temperature. Under the same conditions, the higher the temperature, the faster the gelation. If the room temperature is too high, reduce the amount of Improved Coagulant as appropriate. Conversely, if the room temperature is low, extend the gelation time as needed.
5. This product already contains an appropriate TEMED substitute. If faster gelation is required, additional TEMED may be added as needed immediately before gel preparation.
6. Recommended electrophoresis conditions: 150 V for about 60 min or 200 V for about 45 min.
7. For convenience, opened Improved Coagulant may be stored at 4°C for at least three months.
8. Do not prepare too many gels at one time during gel casting, so that the stacking gel can be added in time. No more than 2-3 gels at a time is recommended.
9. For your safety and health, please wear a lab coat and disposable gloves when operating.
10. It is recommended to refer to the table below when selecting a suitable gel concentration. The table summarizes typical separation ranges of protein molecular-weight markers (10-250 kDa) in SDS-PAGE gels of different concentrations. Actual separation may vary slightly depending on factors such as temperature and pH, so the table is for reference only.

Recommended SDS-PAGE Gel Concentration Guide			
Gel Concentration	Approximate Resolving Range	Primary Use	Comments
6%	50-250 kDa	High-molecular-weight proteins	Suitable for larger proteins
7.5%	30-200 kDa	Medium- to high-molecular-weight proteins	Balanced high-range separation
10%	20-150 kDa	General-purpose protein separation	Commonly used concentration
12.5%	10-100 kDa	Medium- to low-molecular-weight proteins	Suitable for most routine samples
15%	10-70 kDa	Low-molecular-weight proteins	Improved resolution for smaller proteins

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Protocol

Example for preparing one 0.75/1.0/1.5 mm gel

1. Mix equal volumes of Separating Gel Solution 12.5% (2×) and Separating Gel Buffer 12.5% (2×), 2.0/2.7/4.0 mL each. Mix equal volumes of Stacking Gel Solution (2×) and Red Stacking Gel Buffer (2×), 0.5/0.75/1.0 mL each. The detailed gel-casting volumes are shown in the table below.

Separating Gel Formulation			
Gel Thickness	Separating Gel Solution 12.5% (2×)	Separating Gel Buffer 12.5% (2×)	Improved Coagulant
0.75 mm	2.0 mL	2.0 mL	40 μL
1.0 mm	2.7 mL	2.7 mL	55 μL
1.5 mm	4.0 mL	4.0 mL	80 μL
Stacking Gel Formulation			
Gel Thickness	Stacking Gel Solution (2×)	Red Stacking Gel Buffer (2×)	Improved Coagulant
0.75 mm	0.5 mL	0.5 mL	10 μL
1.0 mm	0.75 mL	0.75 mL	15 μL
1.5 mm	1.0 mL	1.0 mL	20 μL

Note: Because of the special physicochemical properties of the dye, mix thoroughly before use.

2. Add 40/55/80 μL of Improved Coagulant to the mixture prepared in Step 1 and mix gently. If gelation is too fast, the amount of Improved Coagulant may be reduced by half. Pour the mixed solution into the gel-casting glass plates until the distance between the liquid level and the top edge of the short glass plate is about 0.5 cm longer than the comb teeth.

Note: This prepared volume is in excess and should not be poured in completely. After adding Improved Coagulant, mix gently to prevent excessive oxygen from entering the gel solution and inhibiting gel polymerization.

3. Add 10/15/20 μL of Improved Coagulant to the mixture prepared in Step 2 and mix gently. Without waiting for the separating gel to solidify, slowly pour the mixed solution into the gel-casting glass plates and insert the comb. This step should be completed within 2 min after pouring the separating gel. The traditional method may also be used, in which the separating gel is overlaid with absolute ethanol before the stacking gel is prepared.

Note: Pour the stacking gel solution slowly to avoid flushing it into the separating gel. After the stacking gel solution is added, gently vibrate the gel stand to level the interface between the stacking gel and separating gel. After adding Improved Coagulant, mix gently to prevent excessive oxygen from entering the gel solution and inhibiting gel polymerization.

4. After the gel has solidified (about 15 min), remove the comb and proceed with electrophoresis.

Note: Use freshly prepared electrophoresis buffer whenever possible. Even if the interface between the stacking gel and separating gel is not perfectly level after solidification, subsequent electrophoresis will not be affected.