SDS-PAGE Gel Preparation Kit (Tris-Gly, 8%)

Introduction

This product provides premixed solutions for stacking and resolving gels suitable for the Tris-glycine electrophoresis system. Simply add the improved accelerator (TEMED-free), pour the resolving gel, and then directly pour the stacking gel without waiting for the resolving gel to solidify—it is simple and fast. The prepared stacking gel is colored, making the loading wells clearly visible and easy to load. The colored dye does not affect subsequent experiments such as electrophoresis, staining, or transfer. The prepared gel is SDS-free and can be used for both denaturing and non-denaturing gel electrophoresis.

This product features one-step gel casting (pour the resolving gel then directly inject the stacking gel without a water overlay), convenient sample loading (the colored stacking gel makes loading wells clearly visible), fast gel preparation (prepare multiple gels in a short time without calculating solution volumes, dilution, or water overlay procedures), clear bands (small molecular weight protein bands are clearer than those in traditional gels), an odor-free formulation (no TEMED, eliminating unpleasant odors), and compatibility with traditional electrophoresis and transfer buffers.

The specifications of this product are based on preparing 0.75 mm thick gels (10 × 8 cm). Each kit can be used to prepare 50 or 125 gels.

Components and Storage

Components	50 T	125 T	Storage
Reagent A (upper glue liquor, 2X)	40 mL	80 mL	4°C Away from light
Reagent B (upper glue buffer, 2X)	40 mL	80 mL	4°C
Reagent C (lower glue liquor, 2X)	120 mL	250 mL	4°C Away from light
Reagent D (lower glue buffer, 2X)	120 mL	250 mL	4°C
Reagent E (PAGE Adhesives)	5 mL	8 mL	-20°C away from light
Shipping: Blue Ice	**Shelf life: 12 months**		

Protocol

The appropriate thickness of PAGE glue preparation method was selected according to the experimental needs, and the gel formula was referred to the attached table.

	Stacking Gel			Resolving Gel		
Gel thickness	0.75 mm	1.00 mm	1.50 mm	0.75 mm	1.00 mm	1.50 mm
Reagent A	0.50 mL	0.75 mL	1.00 mL	-	-	-
Reagent B	0.50 mL	0.75 mL	1.00 mL	-	-	-
Reagent C	-	-	-	2.00 mL	2.70 mL	4.00 mL
Reagent D	-	-	-	2.00 mL	2.70 mL	4.00 mL
Reagent E	10 μL	15 μL	20 μL	40 μL	60 μL	80 μL

Mini glue configuration process (refer to the gel formula and refer to the attached table):

1. Preparation of Resolving Gel (Reagent C and Reagent D, mix at a 1:1 ratio)

   Take equal volumes of Reagent C (lower gel liquor, 2×) and Reagent D (lower gel buffer, 2×) and mix well to obtain the resolving gel premix. Then add the corresponding amount of Reagent E (PAGE Adhesives). Mix appropriately and pour the mixture into the gel casting apparatus. The liquid level should be approximately 0.5 cm lower than the top edge of the short glass plate relative to the comb length (i.e., the distance from the liquid level to the top edge of the short plate should be 0.5 cm greater than the comb tooth length).

***Note:**
1. This solution is excessive, do not inject it all, you can leave a little in the glue cup to judge the solidification state of the glue.
2. After adding Reagent E, it should be mixed gently to prevent too much oxygen from mixing into the gel solution and inhibiting gel polymerization.

2. Preparation of Stacking Gel (Reagent A and Reagent B, mix at a 1:1 ratio)

   2.1 Take equal volumes of Reagent A (upper gel liquor, 2×) and colored Reagent B (upper gel buffer, 2×) and mix well to obtain the stacking gel premix. Then add the corresponding amount of Reagent E (PAGE Adhesives) and mix appropriately.

   2.2 Immediately after pouring the resolving gel (Step 1), without applying a water overlay or waiting for the resolving gel to solidify, slowly add the stacking gel premix onto the resolving gel solution. Be careful not to add too quickly, as this may disrupt the surface of the resolving gel layer. Fill completely, then insert the comb and allow the gel to solidify (approximately 15 minutes). Once the gel has completely solidified, the gel casting procedure is complete, and subsequent electrophoresis experiments can be performed.

   2.3 Gel solidification time is related to temperature. Higher temperatures result in shorter solidification times, while lower temperatures require longer times. The amount of Reagent E (PAGE Adhesives) specified in this manual is determined under standard conditions at 20°C. Adjust the amount accordingly based on the actual temperature.

***Note:**
1. After pouring the resolving gel, inject the stacking gel directly without applying a water overlay.
2. Due to the specific physicochemical properties of the dye, shake Reagent B well before use.
3. After adding Reagent E, mix gently to prevent excessive oxygen from being introduced into the gel solution, which may inhibit gel polymerization.
4. The flatness of the interface between the stacking gel and resolving gel after solidification is slightly lower than that of gels prepared by traditional methods, but this does not affect subsequent electrophoresis.

3. Electrophoresis

   Electrophoresis conditions: 180 V for approximately 60 minutes. When the bromophenol blue dye front has migrated to the bottom of the gel or to the predetermined position of the experiment, the electrophoresis can

be terminated.

FAQs

Question	Possible causes	Answer
The gel is not solidified	1. Coagulant failure 2. The temperature of the glue is low 3. The coagulant is added in the wrong ratio	1. Pay attention to the storage conditions of the coagulant 2. Restore the kit to room temperature for use 3. Use according to the instructions
Missing comb teeth		
The strips are smiling		
The strip is bent	1. When pouring concentrated glue, the speed is faster, and the impact on the separating rubber is greater 2. When inserting the comb, use more force 3. The room temperature is high, and the separation gel solidifies faster	1. When pouring concentrated glue, the action should be gentle 2. When inserting the comb, you should move gently 3. Lower the room temperature, or reduce the coagulation dose, and the minimum should not be less than 0.5%
The sample leaks into the sample well	The concentrated glue dries up, resulting in a gap between the gel and the glass plate	The prepared gel should be used as soon as possible, or stored in a preservation solution at 4°C for one week
The electrophoresis strip is thicker	Less concentrated glue	Increase the length of the concentrate

Note

1. The product specification is based on the glue thickness of 0.75 mm, and the quantity is 50/125T (10 x 8 cm); When the thickness of the glue is 1.0 mm, it can be made into 40/110T (10 x 8 cm); When the thickness of the glue is 1.5 mm, it can be made into 25/70T (10 x 8 cm). The specific number of gels that can be prepared is related to the size of the gel, and there will be errors in the volume of different glue-making equipment.
2. The dosage of PAGE gel coagulant (Reagent E) is for reference only, and the actual dosage can be adjusted according to personal experimental habits and experience.
3. PAGE gel coagulant (Reagent E) should be stored at -20°C, and the storage time at room temperature should be minimized to prevent failure.
4. This product has been added as a substitute for TEMED, and if you need to further accelerate the gel, you can supplement an appropriate amount of TEMED as needed before dispensing.
5. This product is for scientific use only.

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