

SDS-PAGE Gel Preparation Kit (Tricine)

Introduction

Conventional Tris-SDS-PAGE electrophoresis can only resolve large molecular weight proteins effectively and has very low resolution for relatively small molecular weight proteins, especially those below 10 kDa. In contrast, Tricine-SDS-PAGE can excellently separate proteins and peptides in the molecular weight range of 1-10 kDa, making it the primary method for denaturing separation of peptides by electrophoresis.

This product features several significant advantages: it offers ultra-high resolution, capable of resolving molecules as small as 1.2 kDa; the gel casting process is quick, allowing gel preparation in a short time; the included colored stacking gel facilitates convenient sample loading; the improved formulation eliminates the need for a spacer gel; and the gels are SDS-free, enabling their use in non-denaturing electrophoresis as well.

This product includes a complete set of reagents required for Tricine-SDS-PAGE gel preparation. Only distilled water needs to be supplied by the user to prepare high-quality gels of various concentrations with convenient and rapid operation. After electrophoresis, the gels can be directly used for subsequent experiments such as Coomassie Brilliant Blue staining, silver staining, Western blotting, etc. This kit can prepare approximately 30-50 PAGE gels of standard size depending on gel concentration and thickness.

Components and Storage

Components	Size	50 tests	Storage
Reagent A (upper glue liquor, 2x)		40 mL	4°C Away from light
Reagent B (colored upper glue buffer, 2x)		40 mL	4°C
Reagent C (lower glue liquor, 2x)		120 mL	4°C Away from light
Reagent D (lower glue buffer, 2x)		120 mL	4°C
Reagent E (Modified PAGE Adhesives Accelerator)		8 mL	-20°C away from light
Shipping: Blue ice		Shelf life: 12 months	

Protocol

Select the appropriate PAGE upper and lower adhesives according to the experimental needs, and refer to the following table for the formula:

Gel thickness	Stacking Gel			Resolving Gel		
	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 μ L	15 μ L	20 μ L	40 μ L	54 μ L	80 μ L

Take the preparation of a 0.75/1.0/1.5 mm mini glue as an example:

1. Preparing the Resolving Gel

- Take equal volumes of Reagent C (lower glue liquor, 2 \times) and Reagent D (lower glue buffer, 2 \times) and mix well.
- Add the corresponding amount of Reagent E (Modified PAGE Adhesives Accelerator) at a 1% ratio to the above mixture and mix well.
- Add the mixture to the gel casting apparatus until the liquid level is 0.5 cm above the bottom of the short glass plate (i.e., leaving a space 0.5 cm longer than the comb teeth).

***Note:** This solution is excessive. Do not pour all of it into the mold. Leave a small amount in the mixing cup to monitor the gel solidification status.

- (Optional) Overlay the separating gel with an appropriate amount of water or ethanol to flatten the gel surface. After the separating gel has solidified (approximately 15 min), pour off the water or ethanol layer.

***Note:** When a refractive line appears between the water/ethanol and the gel, the gel has solidified.

2. Preparing the Stacking Gel

- Take equal volumes of Reagent A (upper glue liquor, 2 \times) and Reagent B (colored upper glue buffer, 2 \times) and mix well.

***Note:** Due to the specific physicochemical properties of the dye, shake Reagent B well before use.

- Add the corresponding amount of Reagent E (Modified PAGE Adhesives Accelerator) at a 1% ratio to the above mixture and mix well.
- Add the mixture to the gel casting apparatus until it is completely full. Immediately insert the comb with the desired number of wells. Allow the gel to solidify at room temperature for 30 minutes, then carefully remove the comb for subsequent electrophoresis.

***Note:** When adding the stacking gel solution directly onto the separating gel, do not do so too quickly, otherwise it may disturb the surface of the separating gel.

3. Electrophoresis

- Fix the solidified gel into the electrophoresis tank and assemble the electrophoresis apparatus.
- Prepare 1 \times Tris-Tricine-SDS electrophoresis buffer. Fill the inner buffer chamber completely with 1 \times

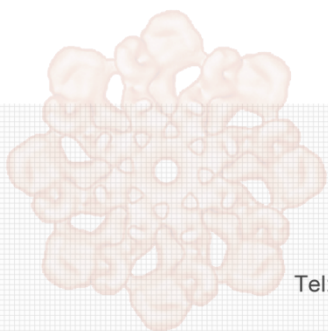
cathode buffer (Running Buffer for Cathode (Tris-Tricine, 5×) (Cat. #: H2011)) to cover the sample wells. Add an appropriate amount of 1× anode buffer (Running Buffer for Anode (Tris-Tricine, 5×) (Cat. #: H2010)) to the outer buffer chamber.

- c. Mix the protein sample with the protein loading buffer thoroughly. Heat at 100°C for 3-5 minutes.
- d. Before loading, gently pipette electrophoresis buffer up and down in the wells to remove any residual gel solution. Load an appropriate concentration and volume of protein sample into the wells.
- e. Electrophoresis conditions: 150 V for approximately 90 minutes, or until the bromophenol blue dye front reaches the bottom of the gel or a predetermined position. For better resolution of small molecular weight proteins, it is recommended to perform electrophoresis at 4°C or in an ice-water bath.

***Note:** After electrophoresis, proceed to subsequent steps as soon as possible to prevent peptide diffusion out of the gel.

■ Note

1. After removing the kit from the refrigerator, allow all reagents to warm to room temperature (above 20°C) before use.
2. Store Reagent E (Modified PAGE Adhesives Accelerator) at -20°C protected from light, where it is stable for 12 months. After opening, it can be stored at 4°C for up to three months. Minimize storage time at room temperature to prevent loss of activity.
3. The recommended amount of Reagent E (Modified PAGE Adhesives Accelerator) is for reference only; the actual amount can be adjusted according to personal experimental habits and experience. Adding more accelerator will speed up gel polymerization, while adding less will slow it down.
4. Gel polymerization time is temperature-dependent. Higher temperatures shorten the polymerization time, while lower temperatures prolong it. Adjust the proportion of Reagent E (Modified PAGE Adhesives Accelerator) according to specific conditions. Regarding high room temperatures, it is recommended to reduce the amount of Reagent E. Conversely, at low room temperatures, extend the polymerization time appropriately.
5. Storage precautions for prepared gels: Avoid repeated and drastic temperature changes. Avoid temperatures below 0°C, as freezing will render the gels unusable. Ensure the gel remains in a hydrated state.
6. The stacking gel prepared with this product has no sample concentrating effect, similar to precast gels. However, compared to traditional PAGE gels, it provides better separation of protein bands.
7. During gel preparation, especially during mixing steps, take care to avoid introducing air bubbles.
8. This product is for scientific research use only.



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