Gradient-like PAGE Gel Rapid Electrophoresis Kit

Introduction

This product is a gradient-like PAGE gel preparation kit. Through a unique premixed formulation for the upper and lower gel layers, it enables broad-spectrum separation capability (10–250 kDa) similar to gradient gels under simplified operation. It allows low-, medium-, and high-molecular-weight proteins to be evenly distributed on a single gel, eliminating the need to select different lower-gel concentrations based on the target protein molecular weight. The kit contains all the components required for gel casting, along with a dedicated electrophoresis buffer powder—ready to use, convenient, and highly efficient.

The product features the following characteristics: ① Adopts a one-step gel casting design—after pouring the lower gel, there is no need to wait for it to polymerize before directly adding the upper gel, eliminating the traditional liquid-sealing step; ② The upper gel is blue, making the loading wells clearly visible and significantly improving the sample-loading experience; ③ Equipped with an improved accelerator that offers good stability and high catalytic efficiency—no additional TEMED is required during gel preparation, completely eliminating the unpleasant odor associated with traditional gel casting; ④ Comes with a dedicated electrophoresis buffer (powder), allowing electrophoresis to be completed in approximately 25 minutes under constant voltage at 200 V, greatly shortening the experimental cycle.

Each kit can prepare approximately 50 mini-gels (0.75 mm thickness). The reagents are valid for 12 months when stored at 2–8°C in the dark, and the accompanying electrophoresis buffer powder can be stored at room temperature for up to 2 years.

Component and Storage

Components	50 T	Storage
Reagent A (upper glue liquor, 2X)	40 mL	4°C away from light
Reagent B (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 (PAGE Adhesives)	5 mL	-20°C away from light
Reagent F (Gradient-Like PAGE Running Buffer, Powder)	10× 500 mL	Room temperature
Shipping: Blue ice	**Shelf life: 12 months**	

Note: Reagent E is recommended to be stored in a dark place at -20°C after dispensing, effective for 12 months.

Opened containers can be stored at 2-8°C for three months. Room temperature storage time should be minimized to prevent failure; Reagent F can be stored at room temperature, with a shelf life of 24 months.

Protocol

1. Gel Preparation

After removing the kit from the refrigerator, place it at room temperature and allow all reagents to warm to room temperature (above 20°C) before use.

Reference Table for Upper and Lower Gel Preparation (using 0.75/1.0/1.5 mm mini-gels as examples):

Gel Type	Reagent	0.75 mm	1.00 mm	1.50 mm
Separating Gel (Lower Layer)	Reagent C	2.0 mL	2.7 mL	4.0 mL
	Reagent D	2.0 mL	2.7 mL	4.0 mL
	Reagent E	40 μL	60 μL	80 μL
Stacking Gel (Top Layer)	Reagent A	0.5 mL	0.75 mL	1.0 mL
	Reagent B	0.5 mL	0.75 mL	1.0 mL
	Reagent E	10 μL	15 μL	20 μL

a. Prepare the separating gel (lower gel): Combine equal volumes of Reagent C and Reagent D (2.0/2.7/4.0 mL each) and mix well. Then add 40/60/80 μL of Reagent E to the mixture and gently mix. Pour the mixed solution into the gel casting glass plates until the liquid level is approximately 0.5 cm above the lower edge of the short glass plate (i.e., 0.5 cm below the top of the short plate, leaving room for the comb).

***Note:** 1. The final prepared solution is in excess; do not pour all of it into the plates—leave a small amount in the mixing cup to monitor the gel polymerization status. 2. After adding Reagent E, mix gently to prevent excessive oxygen from being introduced into the gel solution, which can inhibit polymerization. 3. If more complete separation of low-molecular-weight protein bands is desired, adjust the ratio of Reagent C to Reagent D to 1.1:1 during gel preparation; conversely, for better separation of high-molecular-weight protein bands, adjust the ratio to 0.9:1. 4. The recommended volume of Reagent E is for reference only; the actual amount can be adjusted according to personal experimental habits and experience—adding more accelerates gel polymerization, and vice versa.

b. Prepare the stacking gel (upper gel): Combine equal volumes of Reagent A and Reagent B (0.5/0.75/1.0 mL each) and mix well. Add 10/15/20 μL of Reagent E to the mixture and gently mix. Without waiting for the lower gel to polymerize, gently pour the mixed solution into the gel casting glass plates and insert the comb.

***Note:** 1. Reagent B is colored; due to the special physicochemical properties of the dye, shake the colored stacking gel buffer well before use. 2. Pour the upper gel solution gently to avoid flushing it into the lower gel. After adding the upper gel solution, gently tap the casting stand to level the interface between the upper and lower gels. 3. After adding Reagent E, mix gently to prevent excessive oxygen from being introduced into the gel solution, which can inhibit polymerization. 4. The recommended volume of Reagent E is for reference only; the actual amount can be adjusted according to personal experimental habits and experience—adding more accelerates gel polymerization, and vice versa.

c. After the gel has polymerized (approximately 15 min), it is ready for electrophoresis.

***Note:** 1. After polymerization, the interface between the upper and lower gels may be slightly less even than that of gels prepared by traditional methods, but this does not affect subsequent electrophoresis. 2. Gel polymerization time is temperature-dependent: higher temperatures shorten the polymerization time, and vice versa. Adjust the proportion of Reagent E accordingly; when room temperature is high, reduce the amount of Reagent E appropriately; conversely, if room temperature is low, extend the polymerization time as needed.

2. Electrophoresis

   a. Prepare the electrophoresis buffer (1×): Pour approximately 300 mL of distilled water into a beaker, slowly add 1 pouch of Reagent F while stirring until the particles are completely dissolved, and then bring the volume to 500 mL with distilled water to obtain 1× electrophoresis buffer.

***Note:** This step uses the dedicated rapid electrophoresis buffer. The use of self-prepared Tris-Glycine or other electrophoresis buffers is not recommended.

   b. Secure the prepared gel in the electrophoresis cell and assemble the electrophoresis apparatus.

   c. Add the 1× electrophoresis buffer to the electrophoresis cell: Fill the inner chamber completely with buffer and add buffer to the outer chamber to at least the 1/3 level, but do not exceed the top of the gel plates. Then slowly remove the comb.

***Note:** It is recommended to use freshly prepared electrophoresis buffer, and reuse is not recommended.

   d. Mix the protein samples with denaturing reducing protein loading buffer and heat-treat as required.

***Note:** Recommended denaturing reducing protein loading buffers are K1164 (5×) and K1166 (2×).

   e. Load an appropriate amount of the treated samples into the wells.

***Note:** Before loading, gently pipette the loading wells to remove any residual gel debris. When loading, be careful not to pierce the gel with the pipette tip or insert it too deeply, which could deform the gel plates and cause leakage during subsequent electrophoresis.

   f. Recommended electrophoresis conditions: Constant voltage at 150 V for approximately 35 minutes, or at 200 V for approximately 25 minutes.

3. Transfer: For Western blot transfer, the gels prepared with this product can be operated under the transfer conditions used for conventional 8% PAGE gels.

Note

1. Gels prepared with this product must be used with the dedicated gradient-like electrophoresis buffer. Other electrophoresis buffers cannot be substituted; otherwise, optimal electrophoretic results will not be achieved.
2. During gel preparation, especially during mixing steps, avoid introducing air bubbles as much as possible.
3. This product already contains a TEMED substitute. If further acceleration of gel polymerization is desired, an appropriate amount of TEMED may be supplemented in a fume hood immediately before gel preparation as needed.

4. This product is for scientific research use only.

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