Native-PAGE Gel Preparation and Electrophoresis Kit (PI ≤ 7.0)

Introduction

Native polyacrylamide gel electrophoresis (Native-PAGE) is a polyacrylamide gel electrophoresis on proteins that remain active without adding denaturants such as SDS and phobic ethanol, and is often used for protein identification and purification, such as industrial enzymes. The electrophoresis of natural polyacrylamide gel without SDS can make the biological macromolecules maintain their natural shape and charge during electrophoresis, and their separation is based on the difference of their electrophoretic mobility and the molecular sieve action of the gel, which can obtain higher resolution, especially after electrophoresis separation, it can still maintain the biological activity of biological macromolecules such as proteins and enzymes, which is of great significance for the identification of biological macromolecules.

Various protein molecules have their own isoelectric points due to the different number of basic and acidic amino acids they contain. A low-pH gel system is used for the separation of basic proteins, and a high-pH gel system is used for the separation of acidic proteins. Acidic proteins are usually buffered with a pH of 8.8 in native gel electrophoresis, and the protein will be negatively charged, and the protein will move anode; Electrophoresis for basic proteins is usually carried out in a slightly acidic environment, and the protein is positively charged, so it is necessary to invert the cathode and anode before electrophoresis.

This kit contains all the reagents required for native protein (PI≤ 7.0) gel preparation and electrophoresis, and users only need to bring their own gel preparation utensils and distilled water. This kit can be used to make 30-50 regular-sized pieces of native PAGE gel.

Components and Storage

Components \ Size	50 T	Storage
Acr-Bis (30%, 29:1)	100 mL	4°C away from light
4X Separating Gel Buffer (pH8.8)	100 mL	4℃
4X Stacking Gel Buffer (pH6.8)	50 mL	4℃
APS (Dry Powder)	0.5 g	RT
TEMED	0.5 mL	4°C away from light
5X Loading Buffer (Bromophenol blue)	1 mL	-20℃
5X Electrophoresis Buffer (Dry Powder)	1 L	RT

Shipping: Room Temperature	Shelf life: 12 months

Protocol

1. Bring your own reagents

   Glacial acetic acid, methanol (optional), water, Coomassie brilliant blue staining solution

2. Reagent configuration

   - Preparation of 10% APS: Dissolve 0.5 g of APS dry powder in 5 mL of water, dissolve thoroughly and divide into 1 mL/bottle. Valid for one week at 4°C and 6 months at -20°C.
   - 1×Preparation of electrophoresis buffer: take 5X Electrophoresis Buffer into a 1 L beaker, add about 900 mL of water to dissolve thoroughly, and set the volume to 1L with water, that is, prepare 5X protein electrophoresis buffer. Dilute the 5X protein running buffer to 1X with an appropriate amount of water, such as 200 mL of 5X protein running buffer and add 800 mL of water.
   - Destaining solution configuration (optional): 10 mL of methanol, 10 mL of glacial acetic acid, and 80 mL of water were mixed.

3. Sample preparation

   Add an appropriate amount of loading buffer to the protein sample according to the ratio of protein volume to 5X loading buffer 4:1, for example, add 10 μL of 2X loading buffer to 40 μL of 0.5 mg/mL protein, and load 10 μL (4 μg). No boiling operation is required.

4. Preparation of separating gels

   a. Determine the concentration of the separating gel: select the appropriate gel concentration according to the molecular weight of the target protein, and prepare the separating gel with native PAGE (Table 1 below).

   Table 1: Optimal separation range for different concentrations of PAGE separating gels

PAGE separating gel concentration	Optimal separation range
6%	50～150 kD
8%	30～90 kD
10%	20～80 kD
12%	12～60 kD
15%	10～40 kD

   b. Configure the separating gel components: Mix the ingredients of different volumes in the test tube, and gently stir to mix them well to avoid bubbles (Table 2 below).

   Table 2: The volume of each component required to prepare the gel at different concentrations

Ingredients	Gel concentration (5 mL)

	6%	8%	10%	12%	15%
H2O	2.7 mL	2.4 mL	2.0 mL	1.7 mL	1.2 mL
4X Separating Gel Buffer (pH4.3)	1.25 mL				
Acr-Bis (30%, 29:1)	1 mL	1.3 mL	1.7 mL	2 mL	2.5 mL
100× Separating Gel Accelerator	50 μL				
TEMED	5 μL				
10% APS	50 μL				

c. Fill the gel mold with an appropriate amount of separating gel solution (for mini-gels, the gel solution can be added to about 1.5 cm from the top of the front glass plate or about 0.5 cm from the comb teeth), and then gently cover the separating gel solution with a 1-5 cm layer of water to keep the gel surface flat.

d. Let stand for 15-30 minutes until a clear interface between the gel and the aqueous layer appears, indicating that the gel has polymerized.

***Note:** The polymerization time of the gel is dependent on the ambient temperature. When the temperature is higher in summer, the polymerization is faster; In winter, when the temperature is low, the aggregation time is extended. The amount of APS can be adjusted according to the ambient temperature.

5. Stacking Glut Configuration

a. Mix the ingredients of different volumes in the test tube and mix them with gentle stirring to avoid bubbles (Table 3 below).

Table 3 Stacking gel (5%, 2 mL) configuration required volume of each component

Ingredients	Dosage
H2O	1.15 mL
4X Stacking Gel Buffer (pH6.8)	500 μL
Acr-Bis (30%, 29:1)	330 μL
TEMED	2 μL
10% APS	20 μL

b. Remove the water from the separating gel.

c. Add the prepared stacking gel solution, drain the air bubbles and insert it into the comb.

d. Stand still for 30–60 minutes for the gel to solidify.

***Note:** The polymerization time of the gel is dependent on the ambient temperature. When the temperature is higher in summer, the polymerization is faster; In winter, when the temperature is low, the aggregation time is extended. The amount of APS can be adjusted according to the ambient temperature.

6. electrophoresis

a. Add the configured 1X protein running buffer to the inner tank of the electrophoresis tank and pass it over the loading well.

b. Gently remove the gel comb from the electrophoresis solution, rinse the wells, and then add an appropriate amount of 1X protein running buffer to the outer tank of the electrophoresis tank.

c. Loading: Load a protein with loading buffer, such as 10 μL (4 μg).

d. Electrophoresis: 80 V constant pressure for about 15 min, after the indicator enters the separating gel, replace the 120 V constant pressure, and stop electrophoresis when the indicator moves to the gel bottom plate, about 60 min.

***Note:** Low temperature electrophoresis can be used as an option for electrophoresis, such as an ice bath to reduce temperature-induced protein denaturation. It is recommended that the voltage and electrophoresis time be optimally adjusted according to the electrophoresis results.

e. Staining: Immerse the electrophoresis gel in the Coomassie Brilliant Blue solution (such as InstaBlue Protein Stain Solution, B8226) and stain it by gently shaking at room temperature for 30 minutes.

f. Destaining: Pour out the staining solution, add the decolorization solution directly, and shake slowly at room temperature until the background is clear.

***Note:** If you purchase the InstaBlue Protein Stain Solution (B8226), there is no need for complex operations such as washing, fixing, boiling, and destaining.

Note

1. This kit is suitable for separating proteins with an isoelectric point PI ≤ 7.0. If the isoelectric point PI of the protein is > 7.0, please select the Native-PAGE Gel Preparation and Electrophoresis Kit (PI > 7.0) (K4141).
2. Experimental samples do not need to be heat-treated after they have been added to the loading buffer.
3. During Native-PAGE, the mobility of the protein is not only related to the isoelectric point of the protein, but also to the molecular weight and shape of the protein.
4. During electrophoresis, it is necessary to pay attention to the denaturation of proteins caused by heat caused by excessive voltage, and ice cubes can be placed outside the electrophoresis tank to reduce the temperature.
5. If the molecular weight of the protein is large, the electrophoresis time can be extended so that the protein of interest has sufficient mobility to separate from other proteins, and vice versa.
6. This product is for scientific research use only.

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