# Acidic Protein Non-Denatured Polyacrylamide Gel Preparation Kit

**Cat:** D1260
**Size:** 25 pieces/50 pieces
**Storage:** 2-8°C, away from light, valid for 1 year.

**Kit Components:**

| | 25T | 50T | **Storage** |
|---|---|---|---|
| 30%Acr/Bis(29:1) | 100mL | 100mL×2 | 4℃, away from light |
| 1.5mol/L Tris(pH8.8) | 100mL | 100mL×2 | Room temperature |
| 1.0mol/L Tris(pH6.8) | 30mL | 60mL | Room temperature |
| PAGE Gel Coagulant | 1g | 2g | Dry powder 4℃; Solution -20℃ |
| PAGE Gel Accelerator | 0.8mL | 1.5mL | 4℃, away from light |
| 10×non-denatured electrophoretic buffer | 1L×2 | 1L×4 | Room temperature |

**Introduction:**

The company produces acid protein non-denatured polyacrylamide gel preparation kit is prepared according to the classical method. It is suitable for separating acidic non-denaturing protein. This kit provides various reagents needed to prepare acidic protein non-denatured polyacrylamide gel. Users only need to bring their own gluing apparatus and distilled water to prepare PAGE gel. This kit can prepare about 25/50 pieces of native PAGE gel(that is, non-denatured polyacrylamide gel) with different specifications and different polymerization degrees.

**Protocols(only for reference):**

1. First, add distilled water or deionized water to the dry powder of PAGE gel coagulant(10mL water per gram of PAGE gel coagulant) and configure the solution into 10% solution, and then pack the solution into small volume and freeze it at -20℃, and use it after melting when preparing gel. The validity period of 10%PAGE gel coagulant solution is one week at 4℃.
2. The non-denatured protein polyacrylamide is separated according to the charge of the protein and the molecular weight. Before the experiment, the appropriate concentration of the separation gel is selected and prepared according to the following table. First with the separation glue, and then with the concentrated gel.
3. 1×non-denatured electrophoretic buffer configuration: Take 1 bag of 1L 10×non-denatured electrophoretic buffer, add 800mL deionized water or double steam water to completely dissolve the powder, and then volume with water to 1000mL, that is, 10×non-denatured electrophoretic buffer. Take 100mL 10×non-denatured electrophoretic buffer and add water to 1000mL to obtain 1×non-denatured electrophoretic buffer.

4. The time of running gel is related to the concentration of separating gel and the size of voltage. It is recommended to do pre-experiment or review the literature to select the appropriate voltage to obtain the best separation effect.

| | **Separation gel 15%** | **Separation gel 12%** | **Separation gel 10%** | **Separation gel 8%** | **Concentrated glue 4%** |
|---|---|---|---|---|---|
| Total volume | 10mL | 10mL | 10mL | 10mL | 5mL |
| 30%Acr/Bis(29:1) | 5mL | 4mL | 3.3mL | 2.7mL | 0.67mL |
| 1M Tris-HCl(PH6.8) | 0 | 0 | 0 | 0 | 0.625mL |
| 1.5M Tris-HCl(PH8.8) | 2.5mL | 2.5mL | 2.5mL | 2.5mL | 0 |
| 10%PAGE gel coagulant | 100μL | 100μL | 100μL | 100μL | 75μL |
| PAGE gel accelerator | 10μL | 10μL | 10μL | 10μL | 7.5μL |
| $ddH_2O$ | 2.4mL | 3.4mL | 4.1mL | 4.7mL | 3mL |

**Notes:**

1. PAGE gel coagulant and 10%PAGE gel accelerator are added last, and the reagent added before adding should be mixed.
2. PAGE gel coagulant is volatile. Please close the bottle tightly after use.
3. Generally, the gel can be solidified within 30min at room temperature, such as the temperature is too low, can be put 37℃ temperature box solidification.
4. After filling the separation gel, gently add 1mL $ddH_2O$ to seal the upper layer, and the dividing line can be seen after the gel is solidified.
5. Before filling concentrated gel, pour away the water layer first, and then blot with absorbent paper. Insert the comb as soon as the concentrated gel is poured. After the concentrated gel solidifies, put it into the electrophoresis solution(let the electrophoretic buffer spread over the sample holes), gently pull out the comb to prevent the deformation of the sample holes.
6. The amount of preparation can be added or subtracted according to the above table. If the concentration of gel used is different from the above, it can be adjusted by itself, mainly adjusting the amount of 30% Acr/Bis(concentration×total volume/30%), and finally making up the total volume with water.
7. Sample loading buffer, need to use non-denatured sample loading buffer, add non-denatured sample loading buffer can not boil, mix and then directly sample.