Glycoprotein Gel PAS Stain Kit

Cat: G4835
Size: 3×500mL
Storage:2-8℃, avoid light, valid for 6 months.

Kit Components

Reagent	3×500mL	Storage
Reagent(A):Oxidant	500mL	2-8℃, avoid light
Reagent(B):Schiff Reagent	500mL	2-8℃, avoid light
Reagent(C):Reducing Reagent	500mL	RT, avoid light
Reagent(D):Positive Control	2mg	2-8℃
If theReagent(D):Positive Control needs to be stored for a long time, it is recommended to store it separately at -20°C. Before use, add 2mL of 1×SDS-PAGE loading buffer to a concentration of 1mg/mL and then divide it into 100uL/tube, a total of 10 tubes, leave one tube for use on the same day, and store the rest at -20°C for long-term storage.		

Introduction

Polyacrylamide gel electrophoresis as the most commonly used and effective separation technology, is also widely used in the separation and identification of polysaccharides and their complexes. Nowadays, the methods of polysaccharide staining mainly include PAS staining, Alcian blue staining and fluorescent staining. The principle of PAS staining is to use high iodized acid (HIO4) as an oxidant, destroy the C-C bond of polysaccharide compound structure, the polysaccharide oxidation into polymer aldehyde compounds, the aldehyde compounds generated with Schiff reagent combination, produce purple complex.

Glycoprotein Gel PAS Stain Kit is a convenient, rapid and sensitive colorimetric kit for specific staining of glycoproteins isolated by polyacrylamide gel electrophoresis (PAGE). By treating the gel containing the separated protein with an oxidant, the cis-diol glycan group in the glycoprotein is oxidized to an aldehyde group, which binds to the Schiff Reagent, resulting in a magent-colored chromatographic band.This kit can be used for staining 10-15 mini gels, and its actual detection sensitivity is related to the degree of glycosylation of the target protein.

Self Provided Materials

Methanol, distilled water, and 3% glacial acetic acid solution

Protocol (*for reference only*)

Take the 10% SDS-Page gel of 8×10cm,1.5mm as an example, the procedure are as follows (reagent dosage is for reference only, depending on the amount of completely immersed gel):

Sample dilution

Dilute the sample concentration to 1 mg/ml with 5×loading buffer and the final concentration of SDS-PAGE loading buffer is 1×. Depending on the size of the gel, add 5-10μL of sample per lane and boil at 100°C for 10 minutes before loading.

Gel staining (*See Note 1*)

1. Fixation: Remove the electrophoresed SDS-PAGE gel, add to a vessel containing 50% methanol solution, soak completely for 1h.
2. Wash: Wash the PAGE gel twice with distilled water and shaking by horizontal shaker gently for 10 minutes each time.
3. Oxidation: Transfer the PAGE gel to a vessel containing the Reagent(A): Oxidant and shake by horizontal shaker gently for 1h.
4. Wash: Wash the PAGE gel three times with distilled water and shaking by horizontal shaker gently for 10 minutes each time. (*See Note 3*)
5. Staining: Transfer the PAGE gel to a vessel containing Reagent(B): Schiff Reagent and shake by horizontal shaker gently for 1h.
6. Wash: Wash the PAGE gel twice with distilled water, and shake by horizontal shaker gently for 10 minutes each time.
7. Reduction: Transfer the PAGE gel to a vessel with Reagent(C): Reducing Reagent and shake by horizontal shaker gently for 5 minutes.
8. Wash: Wash the PAGE gel with 3% glacial acetic solution, and the glycoprotein will appear in magenta to

purple-red bands.

9. Storage: Keep the PAGE gel in a 3% glacial acetic acid solution.
10. Photograph: Take a photo as soon as possible and save it. (*See Note 5*)

Result

Glycoprotein, and Positive Control	Magenta to Purple-red bands
Background	Light Pink or Colorless

Note

1. When using reagents, the amount of immersion gel should be added according to the size of the vessel to prevent insufficient reaction or gel drying, which will affect the subsequent test results.
2. The oxidation time should be sufficient, and the optimal temperature of the oxidation is 18-22℃.
3. The oxidized gel must be washed thoroughgoing by distilled water before pouring Schiff Reagent, otherwise the residual oxidant has an oxidation effect on the stain, and the colorless magenta can be oxidized to purple oxidized magenta. A large number of the latter retention in the gel caused a deep background, difficult to elute and affecting the results.
4. The staining time is recommended according to the thickness and size of the gel, and the staining and washing time can be shortened or extended accordingly.
5. Keep the gel in 3% weak acid solution, and the background will color over time. It is recommended to observe as soon as possible and retain the staining results.
6. For your safety and health, please wear experimental clothes and disposable gloves.