Molecular cloning and genetic engineering - SDS-PAGE

Aim

SDS-PAGE is an indispensable procedure for the expression and purification of foreign proteins in E. coli. It can be used to detect the expression of protein (expression level, expression distribution, etc.) and to analyze the purity of the purified target protein.

Material

30% Acrylamide stock solution

1.5mol/L Tris（pH8.8）solution

10% SDS solution

10% Ammonium persulfate

TEMED solution

1.0 mol/L Tris（pH6.8）solution

Coomassie Brilliant Blue R

Decolorizing solution: 10% glacial acetic acid

5×Tris Glycine Electrode Buffer: 15.1g Tris, 94g Glycine and 5g SDS to 1000ml

2×Sample solution: 2% SDS, 5% mercaptoethanol, 10% glycerol, 0.02% bromophenol blue, 0.01mol/L Tris-HCl (pH 8.0) buffer.

Procedure

1. Gel preparation

(1) Install clean glass panels and strips, and fix the glass panels in the electrophoresis tank.

(2) Preparing 10% separating glue: quickly pour separating glue into the gap between two glass plates until the remaining plate width is 1 cm longer than the comb length. Carefully cover the glue with a thin layer of isopropanol or water.

(3) 5% laminated gum is prepared in the process of separation gum polymerization (which can be placed at 37℃ for about 10 min).

(4) After the separation gel is fully polymerized, the isopropanol or water is poured out and the residue on the gel surface is absorbed by filter paper.

(5) Fill the laminated glue and insert a clean comb immediately to avoid bubbles.

(6) After the laminated rubber has been fully polymerized, carefully pull out the comb, remove the plastic strip for sealing, and fix it in the electrophoresis tank.

(7) Adequate electrophoretic buffer is added to the upper and lower electrophoresis tanks.

2. Sample preparation

Add 2x sample solution of equal volume to the protein solution and heat the mixture in a boiling water bath for 5 minutes. The mixture could be sampled after cooling.

3. Load sample

Sampling with a micro-pipette, with each sample added, the amount of sample is determined according to the specific sample concentration, and the unknown concentration is generally 10 microliters.

4. electrophoresis

For one gel, the current in the concentrated gel is set at 15-20 mA. When the dye enters the separating gel, the voltage can be set to 25-30 mA, and the electrophoresis can be continued until the dye reaches 1 cm away from the bottom of the gel.

5. Protein visualization

(1) Dyeing: adding dye solution (the same amount), dyeing at room temperature for 30 minutes to 1h. Tip: Before dyeing, the dye solution can be heated to boil in microwave oven and dyed in shaking machine for about 10

minutes.

(2) Decolorization: The dyed rubber blocks were washed with water three times to remove the surface dyes, and then decolorized by adding decolorizing solution, during which the decolorizing solution was replaced 3-4 times to clear the strips.