Preparation of Denaturing Polyacrylamide Gels and Silver Staining

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Abstract

This is the protocol for the preparation of denaturing polyacrylamide gels and silver staining

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Before start

Prepare:

FORMAMIDE for Acrylamyde (10mL)

- Formamide 9,8mL
  EDTA 0,5M pH 8,0 125µL
  Azul de Bromofenol 1µL
  Xilenocianol 1µL
  1. Vortex in a falcon.
  2. Store in a refrigerator.

- POLYACRYLAMIDE 6% (1L)
  Acrylamide 40% 150mL
  TBE 5X 200mL
  Urea 420g
  MilliQ Water qsp 1 L
  1. Place 200mL of milliQ water together with the TBE and Urea.
  2. Dissolve in shaker under gentle heating.
  3. Add 40% Acrylamide.
  4. Complete for 1L.
  5. Filter with paper filter and store in refrigerator.

- BUFFER FORMAMIDE + BLUE DEXTRAN (600µL)

Blue dextran 100µL
Formamide 500µL

- Blue Dextran (1mL)
  Blue Dextran 50mg
  0.5M EDTA (pH 8.0) 500µL
  Adjust the volume to 1mL and shake until dissolving (stock: 4ºC).

- BIND
  Ethanol-Acetic Acid Solution 1mL
  Bind 3.5µL
  1. Place the Ethanol-Acetic Acid Solution and the Bind in an eppendorf of 1.5.
  2. Mix lightly.
  3. Spread on the plate with a sheet of toilet paper.
  Note: Prepare this solution on time, just leave the Solution Ethanol-Acetic Acid solution ready.

Protocol

PREPARATION OF DENATURING POLYACRYLAMIDE GELS

Step 1.

Step 2.
Clean the surfaces of each plate with acetone using the toilet paper.

Step 3.
Apply 1mL of the methacryloxypropyl trimethoxysilane (PlusOne Bind-Silane; Amersham Pharmacia Biotech) / ethanol-acetic acid (5% glacial acetic acid in 95% ethanol) to the glass plate to adhere the gel.

Note: Use a sheet of toilet paper to spread a solution on the plate.

Step 4.
Apply 1 ml of 2% dimethyldichlorosilane in octamethylcyclotetrasiloxane (PlusOne Repel-Silane ES; Amersham Pharmacia Biotech) to the bigger plate to prevent gel adhesion and allow plaque separation after electrophoresis.

Note: Use a sheet of toilet paper to spread a solution on the board.

Step 5.
Mount both plates facing each other with 0.4mm thick spaces. Attach them with side clips.

Step 6.
Add 60ml of 6% acrylamide gel, 480 µL of 10% ammonium persulfate and 25 µL of TEMED.

Step 7.

Apply the gel between the plates. Avoid blistering until reaching the lower end.

Step 8.
Carefully insert the comb. Allow 1 to 2 hours for complete polymerization.

Step 9.
SAMPLE PREPARATION

Step 10.
Add 8µl of denaturing buffer (0.05% xylenecianol [w / v], 0.05% bromophenol blue [w / v] and 20mM EDTA in formamide) to 2µL of the amplification reaction.

Step 11.
Denature the samples for 5 min at 95 ° C.

Step 12.
Place samples immediately on ice.

Step 13.
PRE-ELECTROPHORESIS AND ELETROPHORESIS

Step 14.
Pre-run in TBE 1X buffer at 120W for 1 h, or until the temperature of the glass plates reaches 55 ° C.

Step 15.
Apply 3µL of each sample to the gel.

Step 16.
Run at 80W for 60-90 minutes at 50 ° - 56 ° C.

Step 17.
SILVER STAINING (Creste et al., 2001 with modifications).

Step 18.
Disassemble gel apparatus, separating the glass plates. The entire staining procedure will be performed with the gel adhered to the smaller plate.

Step 19.
Fix: incubate the gel in 2L of fixative solution (10% ethanol, 1.0% acetic acid) for 10 minutes (shake gently).

Step 20.
Rinse with distilled water for 1 minute.

Step 21.
Pretreatment: Incubate the gel in 2L of 1.5% nitric acid solution for 3 minutes (shake gently).

Step 22.
Rinse with distilled water for 1 minute.

Step 23.

Staining: Impregnate gel with 2L of 0.2% silver nitrate solution for 20 minutes. (shake gently).

Step 24.
Rinse with distilled water for 1 minute.

Step 25.
Develop gel by applying, 1L developing solution (30g / L Na_2CO_3 and 0.54 mL / L 37% formaldehyde) until the bands appear (5 to 10 minutes). Formaldehyde must be added at the time of use. The developing solution should be cooled in a refrigerator.

Step 26.
Stop developing reaction: Incubate the gel in 2L of 5.0% acetic acid solution to stop the development.

Step 27.
Wash the gel rapidly in distilled water. Air dry and photograph