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ProductInformation

REVERSIBLE PROTEIN DETECTION KIT
for membranes and polyacrylamide gels

Product Number **R-PROB**
Storage Temperature 2-8 °C

TECHNICAL BULLETIN

Product Description

The Reversible Protein Detection Kit is suitable for nylon, nitrocellulose and PVDF membranes, and also for PAGE gels. Many protein stains are not suitable for nylon because the positively charged membrane tightly binds negatively charged stains. The sensitivity is similar to that of Coomassie Blue R acrylamide gel staining and is easily reversible with an EDTA solution. This staining kit does not contain cacodylic acid or potassium ferrocyanide. Because the staining procedure is reversible, the blot can be reused for Western blotting.

Reagents

Reagents Provided

Solution A	F 3916	500 ml
Solution B	F 4041	500 ml

Items Required But Not Provided

Fixing Solution F 7264	
OR	
Alternative Fixing Solution	
40% Methanol, 7% Acetic Acid	
Methanol	M 3641
Glacial Acetic Acid	A 6283
EDTA	E 4884
Electrophoresis Grade Water	W 4629

Preparation Instructions

1. Staining Solution
   Immediately before use, mix equal volumes of Solution A and Solution B in a clean container. The resulting staining solution will be a dark purple.
   Note: 50 ml of staining solution is recommended for a small (7 x 8 cm) membrane or gel; 150 ml of staining solution is recommended for a large (13 x 16 cm) membrane or gel.
2. EDTA, 50 mM, pH 8.0 (200 ml)
   Dissolve 3.72 g of EDTA into a beaker containing 150 ml electrophoresis grade water and adjust the pH to 8.0 using 1 M NaOH solution. Adjust the final volume to 200 ml with electrophoresis grade water.
   Note: 100 ml is required for a small (7 x 8 cm) membrane or gel; 200 ml is required for a large (13 x 16 cm) membrane or gel.
3. 10% Acetic Acid

Glacial Acetic Acid	25 ml
Electrophoresis Grade Water	225 ml

Procedure

Protocol for Staining Membranes

1. Washing
   After the protein(s) have been transferred to the membrane, wash the membrane with electrophoresis grade water for 5 minutes. Repeat two times.
   Note: Thorough washing is essential successful staining.
2. Staining
   Incubate the membrane with the staining solution for 3-5 minutes with gentle agitation.
3. Washing
   Decant the staining solution and wash the membrane with electrophoresis grade water for 5 minutes. Repeat two times.
4. Drying
   If the membrane is to remain permanently stained, allow the membrane to air dry.
   (Note: The background stain becomes lighter during air drying).

Protocol for Membrane Stain Reversal

1. Destaining
   Wash the stained membrane from Step 3, above, with EDTA solution for 5-30 minutes until the desired amount destaining is achieved.
2. Washing
   Wash the membrane with electrophoresis grade water for 5 minutes. Repeat two times.

Protocol for Staining Polyacrylamide Gels

(Note: Steps 1 and 2 can be eliminated for non-denaturing gels)

1. Fixing
   After electrophoresis, immerse the gel in Fixing Solution for 20 minutes. Repeat two times.
2. Washing
   Wash the gel with electrophoresis grade water for 30 minutes. Repeat two times.
   (Note: Thorough washing of SDS-PAGE gels is essential for successful staining.)
3. Staining
   Incubate the gel in staining solution for 20-40 minutes with gentle agitation.
4. Washing
   Decant the staining solution and wash the gel with 10% acetic acid until the desired background is obtained.

Protocol for Polyacrylamide Gel Staining Reversal

1. Wash the gel from Staining Polyacrylamide Gels, Step 4 with EDTA solution until the desired amount of destaining is obtained.
2. Wash the gel with Electrophoresis Grade Water or Fixing Solution for 15 minutes. Repeat the washing 2 times or until desired background is achieved.

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