Detection of DIG labeled nucleic acid

Buffers and Solutions:

1. Washing Buffer: 0.1M Maleic acid, 0.15M NaCl, pH7.5; 0.3%(v/v) Tween 20

2. Maleic Acid Buffer: 0.1M Maleic acid, 0.15M NaCl; adjust to pH 7.5 using solid NaOH

   a. Blocking Reagent: Prepare by dissolving Blocking Reagent (bottle 5) in Maleic Acid Buffer to a final concentration of 10% (w/v) with shaking and heating. Autoclave stock solution and store at 2-8°C.

   b. Blocking Solution: Prepare a 1X solution by diluting 10X Blocking Reagent 1:10 with Maleic Acid Buffer. Always prepare fresh.

   i. Antibody Solution: Centrifuge the antibody for 5 minutes at 10,000RPM. Pipette the necessary amount carefully from the surface. Dilute the antibody 1:5000 in Blocking Solution. Can be prepared ahead of time and stored up to 12 hours at 2-8°C.

3. Detection Buffer: 0.1M Tris-HCl, 0.1M NaCl, pH 9.5


Protocol

1. After hybridization, rinse the membrane briefly in Washing Buffer.

2. Incubate for 30 mins in Blocking Solution.

3. Incubate for 30 mins in 20mL Antibody Solution.


5. Equilibrate for 2-5 mins in Detection Buffer.

6. Incubate membrane in 10mL of Color Substrate Solution in the dark. DO NOT SHAKE during color development.

7. Depending on concentration of DIG, color precipitate will start to form after a few minutes and should be complete after 16hrs. Incubation for longer than 16hrs may be necessary to detect lower concentrations of the DIG probe but runs the risk of increased background.

8. Stop the reaction using sterile water or with TE buffer.