

Detection (SKY)

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Reagents

Anti-Mouse IgG [H&L] (Goat) Antibody CY5.5 Conjugated (1mg)

Rockland, Cat. 610-113-121

Antifade (1,4-phenylene-diamine)

Sigma-Aldrich, Cat. P1519, 100 g

Bovine Serum Albumin (BSA)

Roche Diagnostics, Cat. 100-350

Streptavidin Cy5-conjugated (1 mg)

Rockland™, Cat. S000-06

DAPI (4'-6-Diamidino-2-phenylindole)

Sigma-Aldrich, Cat. 18860

Ethyl alcohol, anhydrous

Formamide (FA)

Fluka BioChemika, Cat. 47671

HCl, 1 N

Monoclonal Anti-Digoxin (mouse IgG1), Clone DI-22 (0.1 mg/ml)

Sigma-Aldrich, Cat. D8156

SSC, 20X

Tween 20

Sigma-Aldrich, Cat. P-1379

dH₂O

Preparation

50% Formamide/SSC (FA/SSC)

20X SSC 20 ml

dH₂O 80 ml

Formamide 100 ml

Adjust pH to 7.25 using 1 N HCl

Pre-warm to 45° C

1X SSC

20X SSC 25 ml

dH₂O 475 ml

Pre-warm to 45° C

4X SSC/0.1%Tween 20

20X SSC 200 ml
dH₂O 799 ml
Tween 20 1 ml

Pre-warm to 45° C

Blocking Solution (3% BSA/4X SSC/0.1%Tween 20)

BSA 0.3 g
4X SSC/0.1%Tween 20 10 ml

Vortex until dissolved

Pre-warm to 37° C

DAPI stock solution (f.c.= 0.2 mg/ml)

DAPI 2 mg
dH₂O 10 ml
Aliquot and store at -80°C

DAPI staining solution (f.c.= 80 ng/ml)

DAPI (stock solution) 40 µl
2X SSC 100 ml

Store at 4°C in a light-tight coplin jar.

Procedure

1. Carefully remove rubber cement surrounding coverslips with forceps. Pre-soak slide in formamide/2X SSC if rubber cement is difficult to remove.
2. Wash slides in 50% formamide/2X SSC for 3 x 5 min each, shaking, preferably in 45°C water-bath.
3. Wash slides in 1X SSC for 3 x 5 min, shaking.
4. Dip slides in 4X SSC/0.1% Tween 20; do not let them dry.
5. Add 150 µl of Blocking Solution (3% BSA/4X SSC/0.1%Tween20) to a 24 mm x 60 mm coverslip, invert slide onto coverslip and incubate in a moist hybridization chamber at 37°C for 30 min.
6. Wash slides in 4X SSC/0.1% Tween 20 to wash off blocking solution, 5 min, shaking.
7. Spin all fluorescent dyes for 1 min at 13,000 rpm.
8. Combine the two antibodies, mouse anti-Dig and Streptavidin Cy⁵-conjugated, into the same eppendorf tube, mix well, and apply 150 µl of antibody solution to a 24 mm x 60 mm coverslip. Each antibody should be

diluted 1:200 in 4X SSC/0.1% Tween 20 (see note 4). Invert the slide onto the solution. Incubate the slides in a moist hybridization chamber at 37°C for 60 min.

9. Wash slides in 4X SSC/0.1% Tween 20, 3 x 5 min, shaking.
10. Add 150 µl of the CY5.5 antibody (diluted 1:200 in or 4X SSC/0.1% Tween 20). Incubate slides in a moist hybridization chamber at 37°C for 60 min.
11. Wash slides in 4X SSC/0.1% Tween 20, 3 x 5 min, shaking.
12. Stain slides for 5 min in the DAPI staining solution in a light-protected coplin jar at RT.
13. Wash slides with 2X SSC 3-5 min.
14. Dehydrate slides in ethanol series of 70%, 90%, and 100% for 3 min each. Air-dry slides (protected from light).
15. Apply 35 µl of antifade solution, cover each slide with a 24 mm x 60 mm coverslip, and store in a light-protected container at 4°C until slide is imaged.

Notes

1. Exposure of slides to ambient light should be minimized during all procedures.
2. Carefully remove coverslips during all procedures to minimize scratches.
3. Do not let the slide dry out between washing steps.
4. BSA may contribute to non-specific background, if this is the case, dilute the antibodies in 4X SSC/0.1% Tween 20 (do not use BSA or goat serum).
5. Maintaining correct temperatures for detection washes is important for reducing background.
6. Expiration dates of antibodies require continuous monitoring. Generally, the antibodies stocks should be stored at -30 °C and expire within a year after aliquots are made.
7. The concentration of the antibody dilution can be altered depending on the quality of the antibody.