Detection (FISH)

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

Introduction

Probes labeled with biotin must be detected with fluorescently labeled Avidin, and probes labeled with digoxigenin require detection with a fluorochrome conjugated antibody against this hapten. For example, to detect the biotin labeled probes we routinely use Avidin-FITC, Avidin-TRITC, or Avidin Cy-5. For the probes labeled with digoxigenin, we usually first incubate with mouse-anti-digoxigenin, followed by incubation with sheep anti-mouse Cy5.5, or other fluorochrome conjugated antibodies.

Reagents

Avidin-Cy5
Jackson Immuno Research Lab, Cat. 003-170-083
Avidin-TRITC
Sigma, Cat. A 7169
Avidin-FITC
Vector, Cat. A-2011
BSA (Bovine Serum Albumin)
DAPI
Ethanol, absolute
Formamide
Fluka BioChemika, Cat. 47671
HCl, 1N
Mouse anti-digoxigenin
Sigma, Cat. D 8156
Sheep anti-mouse Cy5.5
Amersham, Cat. RPQ 0115
20X SSC
Tween 20

Preparation of Reagents

50% FA/SSC		final conc.
20X SSC	20 ml	2X
dH_2O	80 ml	
Formamide	100 ml	50%
Total	**200 ml**	

Adjust pH to 7.25 with 1N HCl
Pre-warm to 45°C

1X SSC (for direct labeled probes, i.e., TRITC, FITC or other)

		final conc.
20X SSC	25 ml	1X
dH_2O	475 ml	
Total	**500 ml**	

Pre-warm to 45°C

0.1X SSC (for indirect labeled probes, i.e. Biotin, or Digoxigenin)

		final conc.
20X SSC	2.5 ml	0.1X
dH2O	497.5 ml	
Total	**500 ml**	

Pre-warm to 60°C

4X SSC/0.1%Tween20		final conc.
20X SSC	200 ml	4X
dH_2O	799 ml	
Tween 20	1 ml	0.1%
Total	**1000 ml**	

Pre-warm to 45°C

Blocking Solution (3% BSA/4X SSC/0.1%Tween20)

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

Pre-warm to 37°C

Antibody Solution (1% BSA/4X SSC/0.1%Tween 20)

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

Pre-warm to 37°C

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

DAPI	2 mg
ddH_2O	10 ml

Aliquot and store at -80ºC

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 the rubber cement surrounding the coverslips from hybridized slides.

2. Wash the slides in pre-warmed (45°C) 50% formamide/2X SSC for 3 x 5 min, shaking.

3. Wash slides in pre-warmed (60°C) 0.1X SSC at 60˚C (for indirectly labeled probes) or pre-warmed (45°C) 1X SSC (for directly labeled probes) for 3 x 5 min, shaking.

4. Dip slides in pre-warmed (45°C) 4X SSC/0.1%Tween 20.

5. If using directly labeled probes, wash 3 x 5 min in pre-warmed (45°C) 4X SSC/0.1% Tween 20, and proceed to step 10.

6. Add 120 µl of Blocking Solution (3% BSA/4X SSC/0.1%Tween 20) to the slides and cover them with a 24 mm x 60 mm coverslip. Place slides in a moist hybridization chamber at 37°C for 30 min.

7. Dip slides in 4X SSC/0.15Tween 20 to wash off the excess blocking solution.

8. Add 120 µl of fluorescent antibody (antibody should be diluted 1:200 in 1% BSA/4X SSC/0.1%Tween 20) to the slides, cover with a 24 mm x 60 mm coverslip, and incubate in moist light-tight hybridization chamber at 37°C for 45 min.

9. Wash slides in pre-warmed (45°C) 4X SSC/0.1%Tween 20, for 3 x 5 min, shaking.

10. Stain slides for 2-5 min in DAPI staining solution in a light-protected coplin jar.

11. Wash the slides for 5 min in 2X SSC, shaking.

12. Dehydrate the slides by dipping through an ethanol series of: 70%, 90%, and 100%; air-dry.

13. Apply 35 µl of antifade solution, cover with 24 mm x 60 mm coverslips, store in light-protected container at 4ºC until slide is imaged.

Notes

1. Exposure of slides to ambient light should be minimized during all procedures.

2. Use care in removing coverslips during all procedures to minimize scratches.

3. Spin all fluorescent dyes prior to use for 3 min at 13,000 rpm and carefully pipette the antibody without disturbing the pellet.

4. Do not let the slide dry out between washing steps.