

Modified Fiber FISH detection

This protocol uses a two layer fluorescent + anti-body stacking to maximize DNA fiber detection. After step seven be sure to do washes in the dark, and use a 22 X 60 coverslip for the incubations. (R.T = room temperature). Dissolve first antibody stocks into 1X 4M, then use 1X TNB for the last two detection layers

	<u>Compound</u>	<u>Temp °C</u>	<u>Time (minutes)</u>
0.)	Gently remove rubber cement, place slides into R.T. 2X SSC, allow coverslip to fall off, then begin wash		
1.)	Wash in 2X SSC	R.T.	5
2.)	Wash in 2X SSC	42	10
3.)	Wash in 2X SSC	R.T.	5
4.)	Wash in 1X 4T	R.T.	5
5.)	Incubate in 1X 4M	37	30
6.)	Wash in twice with 1X 4M	R.T.	10
7.)	Incubate FITC-Avidin (1ul/100ul) + Rhod A-DIG (.5ul/100ul) + Mouse A-DIG (1ul/100) in 1X 4M 37C for 30 min (Rhod A-Dig is optional)		
	Wash twice in 1X 4T	R.T	10
8.)	Wash once in 1X TNT	R.T.	5
9.)	Incubate in Biotin-A-avidin (.5ul/100ul) + Dig-A-Mouse (1ul/100ul) in 1X TNB 37C for 30 min		
	Wash 3x in 1X TNT	R.T.	15
10.)	Incubate in FITC Avidin (1ul/100ul) + Rhod A-DIG (2ul/100ul) in 1X TNB 37C for 30 min		
	Wash 3x in 1X TNT	R.T.	15
11.)	EtOH Series (70%-90%-100%)	R.T.	15
12.)	Add 12 ul Vectasheild cover with a 22 x 40 coverslip, squash gently		

Solutions

2X SSC = 100 ml 20X SSC
900 ml ddH2O

5X 4T = 20X SSC
0.25% Tween 20

10X TNT = 1.0 M Tris
1.5 M NaCl
0.25% Tween 20
pH 7.5

5X 4M = 16% Dry bovine milk (Sigma)
20X SSC

5X TNB = 0.5 M Tris (pH 7.5)
0.75 M NaCl
2.5% Blocking reagent (Roche)

50mls

8 g

To volume (heat to dissolve)

25 mls 17 mls H₂O

7.5 mls

1.25 g