

FISH

Probe 1: FITC

Probe 2 : DIG

Dotted lines represent optional overnight steps

Day 1:

Remove gelatin from slides in DEPC-PBS

Incubate slides in DEPC-PBS with $3\mu\text{l}$ H₂O₂/10ml for 15'-30'

Wash slides 3x5' in DEPC-PBS

Let slides air dry (or dry at 55 C)

Incubate slides in Hyb buffer containing the 2 probes overnight @ 65-70 C

Day 2:

Wash slides 1x 10' in 0.2X SSC @ 65-70

2x 25' in 0.2X SSC @ 65-70

Wash slides at room temperature 5' in MABT

Block slides 30'-60' in MABT + 20%HISS + 2% BBR

Incubate 1 hr @ room temperature or overnight @ 4C with 1:500 anti DIG-HRP in blocking buffer

Wash slides 3x 5' in MABT or PBT

Prepare the Tyramide working solution (10 μl of stock in 500 μl of diluent, prepare enough volume to cover your slides)

Incubate 30' in tyramide solution (you can monitor the reaction development under the microscope after 30' to see if you need to leave it longer) (from now on keep slides in dark!!!)

Wash 3x5' in PBS

Inactivate HRP by washing slides 30'-60' in PBS + $3\mu\text{l}$ H₂O₂/10ml

Wash 3x5' in PBS

Block again for 1/2 hr

Add 1:500 anti-FITC-HRP and incubate 1 hr at room temperature or overnight at 4C

Wash slides 3x 5' in MABT or PBT

Prepare the Tyramide working solution (the other color!!!) (10 μ l of stock in 500 μ l of diluent, prepare enough volume to cover your slides)

Incubate 30' in tyramide solution (you can monitor the reaction development under the microscope after 30' to see if you need to leave it longer)

Wash 3x5' in PBS

Mount slides and store in dark