FISH Probes – Automated Hybridization Protocol

Notes

- Protocol can be used with all FISH probes – controls, gene specifics, custom FISH probes.
- Solutions can be made prior to the procedure.
- Further optimization of the protocol may be required.

Required Reagents & Equipment (Not Supplied)

70%, 85%, and 100% EtOH
22x22 mm dimension coverglass
22x50 mm dimension coverglass
Rubber cement
HYBrite or ThermoBrite
Absorbent Material
dH_2O
Wash Solution 1 (WS1) – 0.3% Igepal (Sigma CA-630) or NP-40 / 0.4 x SSC
Wash Solution 2 (WS2) – 0.1% Igepal (Sigma CA-630) or NP-40 / 2 x SSC
DAPI with Antifade

Prep: Pre-soak absorbent material in dH2O to place in HYBrite/ThermoBrite. Prepare wash solutions and warm WS1 to 73°C

Automated Hybridization Procedure:

1. Prepare the program on the HYBrite/ThermoBrite:
    a. For peripheral blood/ bone marrow preparations: Denaturation should be set to 73°C for 2 minutes and 37°C for at least 16 hours.
    b. For FFPE tissue slide preparations: Denaturation should be set to 75°C for 7 minutes and 37°C for at least 16 hours.

2. Place pre-soaked absorbent material in HYBrite/ThermoBrite and ensure HYBrite/ThermoBrite is on and warming.

3. Dehydrate slides:
    a. Place slides in 70% EtOH for 2 minutes.
    b. Transfer slides in 85% EtOH for 2 minutes.
    c. Transfer slides in 100% EtOH for 2 minutes.
    d. Air dry slides.

4. Prepare a probe mixture to apply to slides while slides are drying (2µL of probe and 8µL of buffer for a total of 10µL per cellular area is the recommended mixture).

5. Pipette 10µL of the probe mixture onto the slides over each cellular area and place 22x22 mm coverglass on the slides to cover each cellular area, taking care that there are no air bubbles.

6. Seal the coverglass with rubber cement. Be sure that all edges of coverglass have been sealed with the rubber cement so the probe does not dry out under the coverslip.

7. Place the slides onto the HYBrite/ThermoBrite, close the lid, and begin the program that is specific to your cell type.

8. After incubation period, remove the slides from the HYBrite/ThermoBrite, remove the rubber cement, gently remove the coverglass, and move on to the wash process.

9. Wash Slides:
    a. Place slides in WS1 at 73°C for 2 minutes; agitate slides for the first 10-15 seconds.
    b. Transfer slides into WS2 at room temperature for 2 minutes; agitate slides for the first 10-15 seconds.
    c. Remove the slides and wipe the backside to dry. Allow the rest of the slide to dry in a dark place.

10. Once the slide is completely dry, apply 10µL of DAPI with Antifade to the cellular area and apply the 22x50 mm coverslip. Store slide in a dark place and wait 15-20 minutes before viewing it under the microscope.