*Swift*FISH Rapid Hybridization Buffer Protocol

Notes

- The *Swift*FISH Rapid Hybridization Buffer decreases hybridization time from 16 hours (traditional hybridization buffer) to as little as 1 hour.
- Probe intensity is equivalent to use of traditional hybridization buffer
- Works for the following samples types: peripheral blood (PB), bone marrow (BM) and formalin-fixed paraffin-embedded (FFPE)
- Works with all FISH probes: controls, gene specific, custom FISH probes
- Further optimization of the protocol may be required.

Required Reagents & Equipment (Not Supplied)

Hotplate
70%, 85%, 100% Ethanol
Humidified Slide Chamber
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

Hybridization Setup

1. Take buffer from fridge and pre-warm to 37°C.
2. Mix buffer by tapping/vortexing, making sure there is no precipitate.
3. Place slides on a warm plate (45°C) for 20 minutes.
4. Dehydrate the slides in Ethanol series 70%, 85%, and 100%, 2 minutes each. Let dry.
5. Add 10 µl probe mixture to slide (2 µl probe + 8 µl *Swift*FISH Rapid Hybridization Buffer) using a pipette tip with the end cut.
6. Apply clean 22mm^2 coverslip to slide. Seal edges with rubber cement.
7. Co-denature chromosomes/probe on an hotplate at 72°C for 2 minutes, or 83°C for 5 minutes for FFPE.
8. Place slide in a sealed humidified slide chamber.
9. Incubate at 43°C for 1-2 hours.

Post-Hybridization Washes

1. Pre-warm WS1 (0.3% Igepal (Sigma CA-630) or NP-40 / 0.4 x SSC) for 1 hour to 73°C.
2. Carefully remove the rubber cement around the coverslip.
3. Place slide in WS1 and let stand for exactly 2 minutes.
4. Transfer to WS2 at room temperature for 2 minutes.
5. Let dry in dark.
6. Apply 10 µl DAPI with Antifade and 22mm^2 coverslip.
7. Wait 15-30 minutes then visualize under microscope using the appropriate filter sets.

References

Barch MJ, Knutsen T, Spurbeck JL. The AGT Cytogenetics Laboratory Manual, Third Edition. Lippincott-Raven Philadelphia. 1991.