*Swift*FISH Rapid Hybridization Buffer

Notes

- The SwiftFISH Rapid Hybridization Buffer decreases hybridization time to 2 hours and is also optimized to perform a traditional 16-hour-hybridization.
- Works for the following sample 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.
- Thaw and mix the buffer well prior to use.

Required Reagents & Equipment (Not Supplied)

Thermobrite
70%, 85%, 100% Ethanol
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
18x18mm, 22x22mm, 22x50mm coverslip

Hybridization Setup

1. Take buffer from freezer and bring to room temperature.
2. Mix buffer by vortexing for approximately 10 seconds, making sure there is no precipitate.
3. Place slides on a warm plate (45ºC) for 20 minutes – you can decrease time on the plate by increasing the temperature (i.e. 70ºC for 10 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 SwiftFISH Rapid Hybridization Buffer) using a pipette tip with the end cut if needed as the buffer is thick.
6. Apply clean 18mm^2 or 22mm^2 coverslip to slide. Seal edges with rubber cement.
7. Place slides on Thermobrite/Hybrite with the following setting:
    a. Peripheral Blood/ Bone Marrow Cell Pellets:
        a. 2 Hour Hybridization:
            i. Denaturation: 80-83°C for 2-3 minutes; Hybridization: 37°C for 2 hours.
        b. 16 Hour Hybridization:
            i. Denaturation: 80-83°C for 2-3 minutes; Hybridization: 37°C for 16 hours.
    b. FFPE Tissue Samples:
        a. 2 Hour Hybridization:
            i. Denaturation: 83°C for 5-7 minutes; Hybridization: 37°C for 2 hours.
        b. 16 Hour Hybridization:
            i. Denaturation: 83°C for 5-7 minutes; Hybridization: 37°C for 16 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 73ºC.
2. Carefully remove the rubber cement around the coverslip.
3. Place slide in WS1 and let stand for exactly 2 minutes, agitating for the first ~15 seconds.
4. Transfer to WS2 at room temperature for 2 minutes, agitating for the first ~15 seconds.
5. Let dry in dark.
6. Apply 10 µl DAPI with Antifade and 22x50mm 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.