Circle Slide – Automated Hybridization

Require Reagents & Equipment (Not Supplied)

Circle slides
22x22 mm dimension coverglass
Rubber cement
HYBrite/ThermoBrite
Absorbent Material
dH_2O
Wash Solution 1 (WS1) – 0.3% Igepal (Sigma CA-630) or NP-40 / 0.4 x SSC @ 73°C
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 dH_2O 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. Peripheral blood/ bone marrow preparations: Denaturation should be set to 73°C for 2 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. Drop cell pellet—ensure cells drop throughout both circle wells on the slide. Allow the slides to dry.
4. 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.
5. Prepare probe mixtures to apply to slides while slides are drying (2µL of probe and 8µL of buffer for a total of 10µL per circle well is the recommended mixture). Two different probes can be used on the slide—one in each well. Label your slides appropriately.
6. Pipette 10µL of the probe mixture onto the slides over each circle well and place 22x22 mm coverglass over the wells to cover each circle, taking care that there are no air bubbles.
7. Seal the coverglass with rubber cement. Be sure that all edges of EACH coverglass have been sealed with the rubber cement so the probe does not dry out under the coverslip. (Orange lines represent rubber cement sealing both 22x22 coverslips)

    Example Diagram: 
8. Place the slides onto the HYBrite/ThermoBrite, close the lid, and begin the program that is specific to your cell type.
9. 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.
10. 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.
11. 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

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