Adherent Cell Fixation and Hybridization

Reagents and Equipment:

Fixation Buffer:

- Formaldehyde in 1X Phosphate Buffered Saline (PBS) (final composition is 3.7% formaldehyde in PBS).
- Mix 1mL 3.7% formaldehyde solution, 1mL 10X PBS, and 8mL nuclease-free water.

Wash Buffer A

- Mix 5mL 20X nuclease-free SSC, 5mL deionized formamide, and 40mL nuclease-free water.
- Allow formamide to warm to room temperature (store at 4°C) before opening bottle to minimize oxidation
- Store wash buffer at room temperature, good for months
- Use in chemical fume hood.

Wash Buffer B

- 0.3% Igepal (Sigma CA-630) or NP-40 / 0.4X SSC at 73°C

Wash Buffer C

- 0.1% Igepal (Sigma CA-630) or NP-40 / 2X SSC at room temperature

70%, 85%, and 100% Ethanol
DAPI with Antifade
Multi-Well Cell Chamber
Hotplate at 72-73°C
Humidified Chamber
Incubator at 37°C
22mm^2 Coverslip
Rubber Cement

Fixation Protocol:

1. Grow cells in a multi-well chamber slide.
2. Aspirate growth medium and wash twice with 1mL of 1X PBS per well. Gently tilt chamber slide to wash wells.
3. Add 1mL of fixation buffer and gently tilt chamber slide.
4. Incubate at room temperature for approximately 5-3 minutes.
5. Wash three times with 1mL of 1X PBS.
6. To permeabilize, immerse cells in 1mL of 70% (vol./vol.) ethanol for at least 1 hour at 2-8°C. Cells can be stored at 2-8°C in 70% ethanol up to a week before hybridization.

*Note: This fixation protocol can also be utilized for a 12-well plate system.

Hybridization Protocol:

1. If probe is frozen before using, warm the reconstituted probe solution to room temperature. Mix well by vortexing, then centrifuge briefly. Recommended probe mixture is 2uL of probe and 8uL of hybridization buffer to total 10uL per cellular area.
2. Aspirate the 70% ethanol off the chamber slide with fixed adherent cells.
3. Add 1mL of Wash Buffer A and incubate at room temperature for 2-5 minutes. Aspirate to remove wash buffer.
4. Dehydrate cells through 70%, 85%, and 100%, each for 2 minutes.
5. Apply 10uL of probe mixture to cellular area and cover with a 22mm^2 coverslip. Seal the coverslip with rubber cement.
6. Denature at 72-73°C for 2 minutes in the dark on a hotplate. Incubate in a dark humidified chamber at 37°C for at least 16 hours.
    a. This step can also be done with a Thermobrite/Hybrite as well.
7. Remove from incubator and remove rubber cement.
8. Place slide in Wash Buffer B at 73°C for 2 minutes.
9. Place slide in Wash Buffer C at room temperature for 2 minutes.
10. Allow slide to fully dry and counterstain with DAPI with Antifade.

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