ImageStream® FISHIS ® for Blood Cells

Reagents:

1. Probes: SpectrumGreen Chromosome Enumeration Probes (CEP) for X, Y, or 8 (Abbott Molecular)
2. Carnoy's Fixative (3 parts Methanol : 1 part Glacial Acetic Acid) make fresh, keep cold
3. 2X SSC
4. PBS
5. NP-40

Protocol:

1. **Cell fixation:**
    a. Enrich mononuclear cells from donor blood by ficoll-hypaque density centrifugation OR use cultured cell line
    b. Pellet PBS-washed cells (1-2 X 10^7 cells) in 15 ml polystyrene centrifuge tube (centrifuge 300xg for 8 min)
    c. Resuspend in 1.0 ml ice-cold PBS and transfer to microfuge tube. While vortexing, add 0.4 ml ice-cold 100% Carnoys dropwise. Incubate for 10 minutes at room temperature.
    d. Store at -20C for at least 4 hours and up to 3 months
2. **Hybridization:**
    a. Centrifuge 300xg for 8 min. Carefully remove all of the supernatant with pipet.
    b. Resuspend in 1mL of 0.1%NP-40 in 2X SSC buffer.
    c. Aliquot 50μl (~1-2million) cells per hybridization into siliconized microfuge tubes.
    d. Centrifuge 300xg for 8 min. Carefully remove all of the supernatant with pipet.
    e. Per hybridization reaction, prepare a master mix of 28 μl hybridization buffer (CEP kit hybridization buffer), 2 μl of probe and 10 μl of nuclease-free water.
    f. Resuspend cell pellet in 39 μl of the master mix and transfer to PCR tube
    g. Thermocyler conditions: 5 min 80°C, 9hr 42°C; optional 4°C storage step.
    h. Add 200μl of 0.1%NP-40 in 2x SSC and transfer to siliconized microfuge tube.
    i. Centrifuge 300xg for 8 min.
    j. Resuspend in 200 μl pre-warmed (73°C) 0.3% NP-40/2xSSC.
    k. Incubate at 73°C for 2 minutes, then add 200uL ice-cold PBS
    l. Centrifuge 300xg for 8 min.
    m. Resuspend in 60 μL ice-cold PBS and run on ImageStreamX
3. **Controls:**
    a. <u>Monosomy detection</u>: Spike in known amount of CEP-X probed male into CEP-X-probed female.
    b. <u>Trisomy detection</u>: Spike in known amount of CEP-8+Y-probed male into CEP-8-probed male PBL