RUO
RESEARCH USE ONLY

Instructions For Use
Fluorescence in-situ Hybridization using Pinpoint FISH™ Probes
(60.0°C Wash)

Reagents Provided

- Probe Solution
- FISH Hybridization Buffer

Reagents Not Provided

- 0.4X SSC/ 0.3% NP-40 solution (preparation method provided upon request)
- 2xSSC solution (preparation method provided upon request)
- Antifade Mounting Medium with DAPI

Equipment and Supplies

- In-situ thermocycler instrument (example: Thermobrite, Cytobrite)
- 37°C Incubator (cell culture incubator, or oven)
- Micropipetter and tips appropriate for transferring volumes in the 2 µL to 20 µL range
- Water Bath
- Calibrated Temperature Probe
- Humidity chamber (an airtight container that holds slides and water such that slides will lay flat and not come into direct contact with the water)
- Forceps for slide handling
- Vortex mixer
- Benchtop microcentrifuge
- Container for ice
- 24x50 Coverslips
- 0.6 mL Microcentrifuge tubes or 0.2 mL PCR tubes
- Rubber cement
- 50 mL, 5-well upright glass Coplin jars

Fluorescence Microscope Filter Requirements

Channel	Excitation	Emission
ATTO550/Spectrum Orange	554 nm	576 nm
6-FAM/Spectrum Green/FITC	490 nm	525 nm
TexRed	595 nm	620 nm
Atto643/647N/Cy5	644 nm	669 nm
Atto425/Aqua	436 nm	485 nm

Instructions Using Pinpoint FISH™ DNA Probes

Slide Spotting

1. Spot an aliquot of fixed cell pellet onto a new microscope slide and allow to dry.
    a. Before spotting, submerging new slides in 100% ethanol may help remove any residue that could be present from the slide manufacturing process.
    b. If metaphases are desired, drying should take place in a humidity-controlled chamber to optimize chromosome spreading.
    c. Freshly dropped slides may be artificially aged for 10 minutes in a 90C oven.

Hybridization

1. Prepare hybridization mix for one full slide (30 µL), or in a 3:2 ratio of Hybridization Buffer to probe solution if a smaller volume of hybridization mix is desired.
   Note: Keep probe products on ice and away from full light if not in use, preferably in the dark.
    a. For a 30 µL, full-slide mix add 18 µL of FISH Hybridization Buffer to a PCR tube
    b. For a 30 µL, full-slide mix add 12µL of probe solution
    c. Vortex the tube contents three times, for 5 seconds each time
    d. Centrifuge for 10 seconds
1. Apply hybridization mix to each slide using a micropipetter. Use care not to introduce air bubbles.
2. Apply cover slip.
3. If a bubble is present underneath coverslip, it can be forced out with gentle, even pressure to coverslip.
   Note: Pressing on coverslip can physically damage cells if not done with great care.
4. Seal with rubber cement applied on periphery of coverslip.
   Note: Take great care that no rubber cement gets underneath the slides, it can obstruct slide contact with the heat block during denaturation.
5. Place slides on Thermobrite or Cytobrite and raise slides to 75ºC for 5 minutes.

6. After program ends, carefully move slides into humidity chamber.
7. Incubate slides in humidity chamber at 37ºC for 4-6 hours, or overnight. Hybridization time should not exceed 12 hours.

Postwash

1. Prepare Wash 1: Place 50 mL of 0.4X SSC/ 0.3% NP-40 solution in a Coplin jar and heat using a water bath set to 60.0ºC.
2. Prepare Wash 2: Place 50 mL of 2xSSC solution in a Coplin jar and leave at room temperature.
3. Gently roll off the dry rubber cement seal and remove coverslip. The coverslip can be removed by submersion in a 2X SSC Coplin jar at room temp (safest way), or the coverslip can be gently removed by hand.
4. Ensure temperature of 0.4X SSC/ 0.3% NP-40 solution (Wash 1) inside the Coplin jar is 60.0ºC, then add slides and wait 1 min.
5. Move slides to Wash 2 (2xSSC solution at room temperature).
6. After 1 to 2 minutes, remove slides from Wash 2 and airdry in the dark.
   Note1: Wiping the back of the slides with a clean paper product helps reduce background during imaging but the front and back of slides should be readily distinguishable.
   Note2: Propping up slides on their sides or label-end down will prevent residue such as ink or adhesive potentially running down onto the sample from the label area.
7. Add 18 µL Vectashield with DAPI and apply a coverslip. Use care not to introduce air bubbles.
   Note: Nail polish can be applied around the edges of the coverslip to seal it in place.
8. Let slides sit for 15 minutes.
9. Image slides.