RUO
RESEARCH USE ONLY

Instructions For Use
Fluorescence in-situ Hybridization using Pinpoint FISH™ Probes
(42.0°C Wash)

Reagents Provided

- Probe Solution
- FISH Hybridization Buffer

Reagents Not Provided

- RNase A in 1X PBS, Final concentration 0.5 mg/mL
  Note: This can be reused up to 5 times if stored at 4°C in between uses.
- De-ionized water (dH_2O)
- Series-diluted ethanol (75%, 85%, 100%), prepared using molecular grade ethanol (200 proof) and dH_2O, and stored at -20°C in between uses.
  Note: Ethanol series used after RNase A treatment can be used up to 5X over 1 month.
- 2xSSC solution (preparation method provided upon request)
- PN Buffer (preparation method provided upon request)
- Antifade Mounting Medium with DAPI

Equipment and Supplies

- In-situ thermocycler instrument (Thermobrite or Cytobrite)
- 37°C Incubator (Cell culture incubator, or oven)
- 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)
- Container for ice
- 24x50 Coverslips
- 0.6 mL Microcentrifuge tubes or 0.2 mL microcentrifuge tubes
- Rubber Cement
- 50 mL 5-well upright glass Coplin Jars with screw caps
- Forceps for slide handling

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. Optionally, freshly dropped slides may be artificially aged for 10 minutes in a 90C oven.

Hybridization

1. Place slides in the Coplin jar containing 50 mL RNase A solution at 37ºC, and incubate for 1 hr.
2. Move slides to Coplin jar containing dH_2O for 1 min.
3. Air dry slides in an upright position for 1-2 min.
4. Begin chilled ethanol series:

    Note: Keep Coplin jars at -20 ºC until use.

    a. Place slides in Coplin jar with 50 mL of 75% ethanol for 2 minutes.
    b. Transfer slides to Coplin jar with 50 mL of 85% ethanol for 2 minutes.
    c. Transfer slides to Coplin jar with 50 mL of 100% ethanol for 2 minutes.
5. Allow slides to airdry completely.
6. Prepare hybridization mix for one full slide (30 µL).
    a. In a microcentrifuge tube add 18µl of FISH Hybridization Buffer
    b. Add 12µl of probe product
    c. Vortex the contents three times, for 5 seconds each time
    d. Centrifuge for 10 seconds.

7. Apply 30µL of hybridization mix to each slide in one even drop using a micropipetter. Use care not to introduce air bubbles.
8. Apply cover slip.
9. If an air bubble is present underneath the coverslip, it can be forced out with gentle, even pressure to the middle of the coverslip.
   Note: Pressing on coverslip can physically damage cells if not done with great care.
10. 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 heated block of the in-situ thermocycler instrument.
11. Place slides on Thermobrite or Cytobrite and bring them to 75ºC for 5 minutes.
12. After program ends, carefully move slides into humidity chamber.
13. Incubate slides in humidity chamber at 37ºC for 4-6 hours, or overnight. Hybridization time should not exceed 12 hours.

Postwash

1. Place 50 mL of 2X SSC solution in a Coplin jar and heat using a water bath set to 42.0ºC.
2. Also place a bottle with at least an additional 200 mL of 2X SSC solution in the water bath.
3. Place 50 mL of PN Buffer in a Coplin jar and leave at room temperature.
4. Taking the slides from the humidity chamber, gently roll off the dry rubber cement seal and remove coverslip by submersion in 2X SSC in a Coplin jar at room temp. Gently agitate, if necessary, to dislodge coverslip.
5. After confirming the correct 2X SSC temperature with a calibrated thermometer, place slides in the Coplin jar with 2X SSC at 42ºC for 5 minutes.
6. After 5 minutes, dump out the Coplin jar's contents, holding the slides in place with a gloved hand. Refill the jar immediately with a fresh 50 mL of warm 2X SSC from the bottle and return it to the bath.
7. Repeat for a total of 5 washes, lasting 5 minutes each.
8. At the end of the fifth 5-minute wash, transfer the slides to the PN buffer for 1 minutes.
9. Without allowing slides to dry completely, add 18 µL 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.
10. Let slides sit for 15 minutes.
11. Image slides.