KromaTiD

Fluorescent In-Situ Hybridization (FISH) with KromaTiD Pinpoint FISH Probes

MATERIALS:

Reagents: (preparation and storage methods provided where indicated)

- RNase A in 1X PBS (Qiagen catalog: 19101) Final concentration 0.5mg/mL.
  Note: this can be reused up to 5 times if stored at 4ºC in between uses
- FISH Hybridization Buffer (provided)
- Molecular grade (RNase/DNase free) H2O
- Two sets of series-diluted ethanol (75%, 85%, 100%), prepared in separate Copelin jars and stored at -20ºC in between uses. Prepare using molecular grade ethanol (200 proof) and ddH20
  Note: The ethanol series used after RNase treatment can be used up to 5X and stored at -20ºC in between uses for up to 1 month. The series used after formamide treatment should be discarded after each use due to the introduction of formamide.
- 70% Formamide/ 2X SSC Solution (Sigma Aldrich: ACS reagent 99.5% catalog: 221198-1L)
  Note: this can be reused up to 5X if stored at 4ºC in between uses
- PN Buffer (preparation method provided upon request)
- DAPI (Vector Laboratories catalog: H-1200)
- 0.4X SSC/ 0.3% NP-40 solution (preparation method provided upon request)
- 2xSSC/0.3% NP-40 solution (preparation method provided upon request)

Equipment:

- 37ºC Incubator (Cell culture incubator, or oven with the slide in a humidity chamber)
- Circulating Water Bath
- Temperature Probe

Supplies:

- 24x50 Coverslips
- 0.6 mL Microcentrifuge tubes or 0.2 mL PCR tubes
- Rubber Cement

- 8-12 50 mL 5 well upright glass Copelin Jars with screw caps
- Forceps for slide handling
- Kimwipes

METHOD:

1. Heat 50 mL of 70% Formamide/2X SSC solution in a Copelin jar using a water bath set at 73.5ºC. Place temperature probe inside jar and cover the top of the jar (suggest using lid set on top to cover the remainder of the opening).
2. Place slides in the Copelin containing the RNase A solution, and incubate the jar for 1 hr. at 37ºC.

   Note: Slides can be placed back to back, two per well in the jar for the internal wells, one per well for the external wells (sample-side facing inward rather than toward the glass).
3. Place slides in Copelin jar containing dH_2O for 5 min.
4. Air dry slides upright on paper towel (suggest propping against a tip box).
5. Begin chilled ethanol series:
   Note: this can be done at room temperature if the Copelin jars for the series are kept at -20 ºC until use.
   a. Place slides in Copelin jar with 75% ethanol for 2 minutes.
   b. Transfer slides to Copelin jar with 85% ethanol for 2 minutes.
   c. Transfer slides to Copelin jar with 100% ethanol for 2 minutes.
6. Air dry slides upright on a paper towel.
7. Ensure temperature of formamide/ 2X SSC solution inside the Copelin jar is 73ºC.
8. Place slides in heated formamide/ 2X SSC solution for 6.5 minutes.
9. Remove slides from formamide and **IMMEDIATELY** transfer into chilled 75% ethanol and proceed with remainder of ethanol series per step 5.

   Note: this ethanol series can only be used once due to the introduction of formamide from the previous step.
10. Prepare hybridization mix for one full slide.
    a. In a PCR tube add 18µl of FISH Hybridization Buffer
    b. Add 1µl of CEP17 Centromeric Probe
    c. Add 1µl of TP53 Probe
    d. Add 10µl of dH_2O (molecular grade)
    e. Pipette up and down 5X and vortex for 10 seconds
    f. Centrifuge for 10 seconds.
    g. Incubate hybridization mix at 75ºC for 5 minutes

h. Immediately transfer to ice

11. Slowly apply hybridization mix (30µl) to dry slide in one even drop, using a pipette. Use care not to introduce air bubbles.
12. Apply cover slip.
13. If an air bubble is present underneath the coverslip, apply gentle, even pressure to the middle of the coverslip until the air bubble has been forced out of the interface.
14. Seal with rubber cement (apply evenly on periphery of coverslip to seal) and incubate at 37ºC for 4-6 hours, or O/N. Do not allow hybridization time to exceed 12 hours.
15. Wash 1: Prepare 50 mL of 0.4X SSC/ 0.3% NP-40 solution in a Copelin jar and heat using a water bath set at 73.5ºC, with care not to submerge lid area. Place temperature probe inside jar and cover the top of the jar (suggest using lid set on top to cover the remainder of the opening).
16. Wash 2: Prepare 50 mL of 2xSSC/0.3% NP-40 solution in a Copelin jar and keep at room temperature.
17. Gently roll off the dry rubber cement seal, and remove coverslip by submersion in 2X SSC in a Copelin jar at room temp. Gently agitate if necessary, to dislodge coverslip.
18. Ensure temperature of 0.4X SSC/ 0.3% NP-40 solution inside the Copelin jar is 73ºC.
19. Place slide into Wash 1 (heated 0.4X SSC/ 0.3% NP-40 solution) for 2 min.
20. Remove slide immediately from Wash 1 and place directly into Wash 2 (2xSSC/0.3% NP-40 solution at room temperature).
21. After 1 to 2 minutes, remove slide from Wash 2 and wipe back of slide with a clean kimwipe.
22. Place slide upright on paper towel to dry in the dark.
23. Add 18 ul Vectashield with DAPI and apply a coverslip. Use care not to introduce air bubbles. Let slide sit for 15 minutes.
24. Image slide.