Fluorescence In Situ Hybridization Protocol (for biotinylated probes)

- Denature the chromosomes

  a. Treat the slide with 70% Formamide, 2XSSC for 2 minutes at 70°C.

  b. Dehydrate the slide through 70%, 85%, and 100% ethanol for one minute each.

  c. Air-dry and pre-warm the slide at 37°C on a slide warmer prior to hybridization.

- Denature the Probe

  a. Mix 7 ul of hybridization buffer with 3 ul probe mixture in a 0.5 ml tube.

  b. Denature the hybridization mix at 70°C for 5-10 minutes.

- Hybridization

  a. Put the hybridization mix onto the slide and apply a 22x22mm coverslip.

  b. Seal the edge of the coverslip with rubber cement.

  c. Keep the slide in a moist chamber overnight at 37°C.

- Fluorescence staining

  a. Peel off the rubber cement and take off the coverslip.

  b. Wash the slide with 50% formamide, 2X SSC at 45°C three times for 5 minutes.

  c. Wash the slide with 4X SSC, 0.05% Tween20 three times for 2 minutes.

  d. Wash the slide for 2 minutes in 4X SSC at room temperature.

  e. Put 100 ul of Avidin (5 ug/ml Avidin in PNM buffer) onto the slide and apply a coverslip.

f. Incubate the slide for 20 minutes at room temperature in a DARK moisture chamber.

g. Repeat steps (c) and (d)

h. Put 100 ul anti-Avidin (1:200 dilution in PNM buffer) and apply coverslip.

i. Incubate the slide for 20 minutes at room temperature in a DARK moisture chamber.

j. Repeat steps (c), (d), and (e), followed by step (c) and (d).

k. Put 40 ul of antifade solution (0.5 ug/ml PI or DAPI as counterstain) onto the slide and apply coverslip.

- Examine the slides or store in the Dark

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