Catalog #	Mouse Chr	Format	Clone
IDMF7000	8	FITC	8.4a

FISH Short Protocol

Hybridization

1. Chromosomes are prepared following standard protocols.
2. Glass slides are acid-cleaned and stored in deionized water. Drain the excess of water on the slides and drop the chromosome suspension.
3. After air-drying age the slides at 50-55 °C for 3 hours.
4. Prepare the labeled probe in hybridization buffer (50% formamide / 2XSSC pH 6.8 / 10% dextran sulphate). For the FITC labeled probe take 5.0 µl of probe and add 5.0 µl of hybridization buffer to make a total volume of 10µl.

   Note 1 : Probes are labeled directly with a fluorochrome. During hybridization and post-hybridization procedures, minimize exposure to light of tubes and slides with the probe.

5. Apply 10µl of diluted probe onto the slide and cover with a 22X22 mm coverslip and seal with rubber cement.
6. Allow evaporation of the rubber solution and denature on a hot plate the chromosomes/probe at 68.3°C for 5 minutes, and hybridize for 12-16 hours in a humidified chamber at 37°C.

Post-hybridization washings:

1. Prepare a “0.4X” solution containing 0.4XSSC with 0.3% Igepal (Sigma) pour in a coplin jar and warm up to 73°C in a water bath. Allow about 2 hours until complete equilibration of the temperature in the jar.
2. Then, carefully remove the rubber cement from the slides and place them in a coplin jar with “2X” solution containing 2XSSC and 0.1% Igepal at room temperature. Periodically gently shake to remove the coverslips.
3. Wash the slides in the hot 0.4X solution for 2 minutes, then very carefully transfer them to the 2X solution and incubate at room temperature for 1 minute.
4.

   Note 2: Wash one slide at the time and allow an interval between slides of at least 3 minutes to re-establish the temperature on the hot solution.

5. Finally, rinse briefly the slides in double distilled water and air dry.
6. Mount with Vectashield/DAPI.
7. Proceed with microscope analysis using the appropriate wavelength for the fluorochrome used.