Mouse & Rat IDetect™ Chromosome Paint Probes
FISH Protocol

Preparation of the Solutions

PBS

Prepare PBS by diluting 20 ml of PBS 10X (ID Labs Cat # IS1125-10) with 180 ml of purified H_2O. Store at ambient temperature. Discard stock solution after 6 months, or sooner if solution appears cloudy or contaminated.

Pepsin Stock and Working Solutions

Prepare a 10% Pepsin stock solution by mixing 0.1 gram pepsin in 1 ml ddH_2O (pre-warmed to 37 ℃). Aliquot into 25 µl volumes and store at -20 ℃ until ready to use. When needed, prepare a 0.005% pepsin working solution by mixing 25 µl of the 10% pepsin stock into solution of 49.5 ml ddH_2O and 0.5 ml 1.0 N HCl that has been warmed to 37 º C in a glass jar (using a 37 ℃ water bath).

Formaldehyde Fixation Solution

Mix 12.5 ml of 10% neutral buffered formalin, 37 ml of 1X PBS (ID Labs Cat # IS1125-01), and 2.5 ml of 1M $MgCl_2$.

Slide Pretreatment for FISH (For Metaphase and / or Tissue Sections)

1. Take prepared / dropped slides from the freezer.
2. Allow slide(s) to completely dry at room temperature.
3. Place the slides in 2X SSC, pH7.0 for 2 minutes at 73℃
4. Transfer slide(s) into the **0.005 % Pepsin Working Solution** (prepared above) for 10 minutes at 37 ℃.
5. Wash slide(s) in 1X PBS for 5 minutes at room temperature.
6. Fix slides in 1% formaldehyde for 5 minutes at room temperature.
7. Rinse slides in 1X PBS with **a few drops of 1M glycine, pH 8.5, added (~100 µl per 50 ml PBS),** for 5 minutes at room temperature.
8. Dehydrate slide(s) by immersing sequentially for 1 minute each in 70%, 85% and 100% ethanol solutions at room temperature.
9. Proceed with the appropriate FISH protocol.

A1. Probe preparation

1. Dilute the probe
    a. For **IDetect™ Mouse Chr Y Probe IDMB1055, mix 5 µl of probe and 5 µl of Hybridization Buffer (supplied)**
    b. For IDetect™ **Rat** Probes, **mix 5 µl of probe and 5 µl of Hybridization Buffer (supplied)**

2. Apply 10 µl of diluted probe onto the slide and cover with a 22X22 mm coverslip and seal with rubber cement.
3. Allow evaporation of the rubber solution and co-denature probe and chromosomal DNA on a hot plate the chromosomes/probe at **69°C for 2 minutes**

(Note 2: Please note new denaturation time of 2 minutes)

A2. Hybridization of IDetect™ Probes

1. Hybridize at 37-42°C in a humidified chamber:
    a. For IDetect™ **Mouse** Probes, hybridize **4-16 hours**
    b. For IDetect™ **Rat** Probes, for Chromosome X Probes, hybridize **overnight**. For Chromosome Y Probes, hybridize **4-16 hours.**

A3. Post hybridization Washes

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. 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.

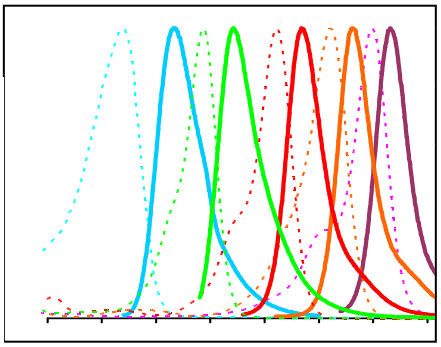
4. Wash one slide at a time and allow an interval between slides of at least 3 minutes to re-establish the temperature on the hot solution
5. Rinse the slides briefly in ddH_2O and air dry.
6. Mount with Vectashield/DAPI.
7. Proceed with microscope analysis using the appropriate wavelength filter for the fluorochrome used.

NOTE: Optimal Dilutions and reaction conditions must be determined by the end user.

An alternative FISH Protocol, with separate probe and chromosome denaturation, for IDetect™ Probes, is available upon request.

IDetect™ FISH probes in 5 colours from ID Labs™

Colour	Fluorochrome	Ex. (nm)	Em. (nm)
Aqua	IDYE™ 415	418±15	467±10
Green	IDYE™ 495	493±10	521±10
Red	IDYE™ 556	548±15	573±20
Orange	IDYE™ 616	611±10	631±15
Far-Red	IDYE™ 647	653±10	672±10



ID Labs References:

Mouse IDetect™ Probes:

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- Zhang, S., et al. "Fusion of human hematopoietic progenitor cells and murine cardiomyocytes is mediated by α4β1 integrin/vascular cell adhesion molecule-1 interaction." Circ Res. Vol 100. pp. 693-702. 2007
- Hu, B., et al. "Bone marrow cells can give rise to ameloblast-like cells". J Dent Res. Vol 85(5). pp. 416-421. 2006.
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- Tankimanova, M., et al. "The initiation of homologous chromosome synapsis in mouse fetal oocytes is not directly driven by centromere and telomere clustering in the bouquet". Cytogenet Genome Res. Vol 105. pp.172-181. 2004.
- Hansford, L., et al. "Mechanisms of embryonal tumor initiation: Distinct roles for MycN expression and MYCN amplification". PNAS. Vol 1(34) pp. 12664-12669. 2004.

Rat IDetect™ Probes:

- Jai, W., et al. "Tumor-infiltrating, myeloid derived suppressor cells inhibit T cell activity by nitric oxide production in an intracranial rat glioma + vaccination model". Journal of NeuroImmunology. Vol 475038. 2010.
- Tabar, V., et al. "Migration and differentiation of neural precursors derived from human embryonic stem cells in the rat brain". Nature Biotechnology. Vol 23(5). Pp. 601-606. 2005.

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