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Mouse 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 (Sigma cat # P7012) in 1 ml ddH_2O (pre-warmed to 37 ℃). Aliquot into 25 µl volumes and store at -20 ℃ until ready to use. Just before use, prepare a 0.005% pepsin working solution by mixing 25 µl of the 10% pepsin stock into a solution of 49.5 ml ddH_2O and 0.5 ml 1.0 N HCl that has been warmed to 37 ºC in a glass jar (using a 37 ℃ water bath).

Formalin Fixation Solution (2.5%)

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 $MgCl_2$ (EMD cat # MX0045)

IDetect™ FISH Probes are tested by ID Labs Inc. on blood metaphase / interphase spreads only. While many labs have used our IDetect™ FISH Probes successfully on paraffin and frozen sections, cytospins, sperm samples, etc., our technical support is limited to applications involving blood metaphase / interphase spreads at this time.

Slide Pretreatment for FISH (For Metaphase Spreads)

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 2.5% formalin for 5 minutes at room temperature.
7. Rinse slides in 1X PBS with **a few drops of 1M Glycine** (Bioshop cat # GLN001)**, 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

For IDetect™ **Mouse** Probes, **mix 3 µl of probe and 7 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 (**37°C is optimal**):

For IDetect™ **Mouse** Probes, hybridize **4-16 hours**

A3. Post hybridization Washes

1. Prepare a "0.4X" solution containing 0.4XSSC with 0.3% Igepal (Sigma cat # 542334) 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.

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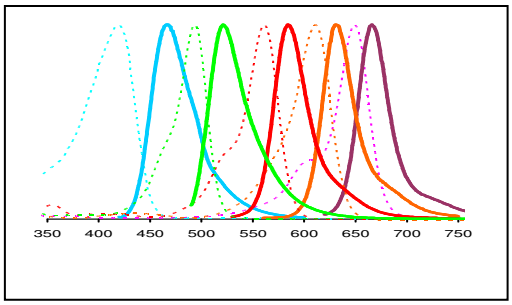
2. Carefully remove the rubber cement from the slides and place them in a coplin jar with "2X" solution containing 2XSSC and 0.1% Igepal at room temperature. Periodically gently shake to remove the coverslips.
3. Wash the slides in the hot 0.4X solution for 2 minutes, then very carefully transfer them 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.

GUIDING NOTES:
We only test our IDetect™ FISH Probes on blood metaphase / interphase spreads. Any other techniques will have to be optimized by the investigator. Optimal Dilutions and reaction conditions must be determined by the end user.
DAPI excites at about 360 nm and emits at about 460 nm when bound to DNA, producing a blue fluorescence. DAPI may also stain RNA.
When using Aqua (IDYE™ 415) Labeled IDetect™ Probes, for best visualization we recommend using a DAPI concentration of 75 ng/ml. The DAPI should be diluted with Mounting Media.
When using Red (IDYE™ 556) or Green (IDYE™ 495) Labeled IDetect™ Probes, for best visualization we recommend using a DAPI concentration of 150 ng/ml. The DAPI should be diluted with Mounting Media.

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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For In Vitro Research Use Only.