

# Fluorescence *IN SITU* Hybridisation Protocol for Whole Chromosome Paints



## Introduction

The FISH protocol is divided into two stages. Denaturation and Hybridisation are performed on **Day One**. Washing and Detection are performed on **Day Two**.

On day one, the DNA of the chromosomes and paints is denatured and the hybridisation process (reannealing) takes place overnight. On day two, the slides are washed to remove unbound DNA sequences followed by detection, counterstaining and mounting.

## Requirements (not provided)

Equipment	Reagents
Ethanol cleaned slides	Sodium Chloride
Coverslips	Sodium Citrate
Eppendorf tubes	HCl
Coplin jars	Pepsin
Humidified chamber	Formamide
Micro-pipette 1µl, 10µl, 500µl	Absolute Ethanol
Pipette 10ml, 20ml	Acetic Acid
Vortex	Double Distilled Water
Parafilm	Fixogum Rubber Cement
Micro-centrifuge	
45°C, 65°C Water bath	
37°C Incubator	

### Approx time:

Slide preparation 30 min + overnight or + 60 min  
Denaturation and hybridisation: 30 min + overnight

**Note:** *Metaphase spreads on slides can be prepared on previous day and aged overnight at room temperature, or on day one and aged for 60 min on hot plate.*

**This product is for research use only**

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## Denaturation and Hybridisation – Day One

**Solutions to be prepared:** 20XSSC  
2XSSC  
Denaturation solution  
Pepsin solution (optional)

**Solution 20XSSC:** 87.6g NaCl  
44.1g Na Citrate  
up to 500ml Deionised Distilled water  
*Adjust pH to 7.0 using concentrated HCl (before finalising water volume), aliquot and autoclave. Store at 4°C).*

**Solution 2XSSC:** 50ml 20XSSC  
450ml Deionised distilled water  
500ml 2XSSC

**Denaturation solution:** 70ml Formamide  
30ml 2XSSC  
100ml Denaturation Solution

**Pepsin solution:** 50µl Pepsin solution (1% in water)  
49.5ml 10mM HCl  
50ml Pepsin Solution  
*Stock pepsin solution can be stored at -20°C in small aliquots.*

**Note:** *Ensure all solutions are mixed well.*

*All solution volumes sufficient for 10 slides*

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## Procedure: Probe preparation & denaturation

1. Warm to 37°C, vortex and centrifuge for 1-3 seconds.

**Note:** *There are two kinds of paints:*

- *Ready to Use (RTU): use 15µl per slide*
- *Concentrated paint (CONC): add 3µl of chromosome paint to 12µl of Hybridisation Buffer.*

*(For dual colour painting, add 3µl of each concentrated paint to 9µl of hybridisation buffer)*

*Use a total of 15µl for a 34x22 slide, 10µl for a 22x22 slide*

2. Denature probe for 10min at 65°C, and hold at 37°C for 30-60min.

## Pepsin treatment (optional):

**Note:** *Pepsin treatment replaces steps 3 and 4 of Slide Preparation and Denaturation.*

Slides can be treated on previous day:

Drop metaphase onto clean slide and dry at room temperature.

Check slide with phase contrast microscope.

Dehydrate slide in 100% ethanol for 5 min and dry at room temperature.

Incubate slide in pepsin solution for 2-5 min (depending on amount of cytoplasm).

Wash in 2XSSC for 1 min. Repeat twice and rinse briefly in distilled water.

Dehydrate by serial ethanol washing for 2 min each in 70% (v/v) ethanol, 70%, 90%, 90%, and 5min 100%. Dry at room temperature.

## Procedure: Slide preparation & denaturation

3. Prepare new slides with fresh metaphase spreads, which have been fixed with 3:1 methanol:acetic acid.

**Note:** *If cytoplasm is still present on the slides treat the slides with pepsin (see Optional pepsin treatment)*

4. Dehydrate by serial ethanol washing for 2 min each in 70% (v/v) ethanol, 70%, 90%, 90%, and 5min 100%. Age for 60 min at 65°C.

**Note:** *If you have pre treated the slides a day in advance and age them overnight at room temperature you do not need to age for 60 min on 65°C hot plate.*

5. Denature slide by incubating in pre-warmed Denaturation solution at 65°C for 1½ - 2 min.
6. Quench slides in ice-cold 70% (v/v) ethanol for 4 min and dehydrate by serial ethanol washing for 2 min each in 70% (v/v) ethanol, 70%, 90%, 90%, and 5 min 100%. Dry at room temperature.

**Note:** *Denaturation of the slides is an important step. Be sure that Denaturation solution is at the right temperature. Some slides benefit from 1½ min denaturation and others up to 2 min. The right timing, which is determined by trial and error, depends on type of cells used, metaphase preparation, brand of formamide, etc.*

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## **Procedure: Hybridisation**

7. Apply probe (15µl\*) onto the slide. Apply coverslip and remove air bubbles by gently pushing on coverslip with a pencil. Seal with rubber cement.
8. Place slide in an air tight, prewarmed humidified chamber and incubate overnight in the dark at 37°C.

**Note:** *With good quality metaphase spreads a minimum period of 4 hours can be sufficient for hybridisation. \* see note of step 1.*

## **Proceed with detection protocol**

**Note:** *Post hybridisation washes are incorporated in the Detection Protocol as part of Day 2.*

*Cambio recommend the use of their Detection and Amplification kits to get the best results possible with these products. Copies of all of our protocols are available on our website [www.cambio.co.uk](http://www.cambio.co.uk)*

Biotin Labelled Paints	
1043-KB-50	Detect using Cy3
1596-KC-50	Detect using Cy5
1082-KT-50	Detect using Texas Red
1089-KB-50	Detect using FITC

FITC Labelled Paints	
1084-KF-50	FITC Amplification Kit

Cy3 Labelled Paints	
Cy3-09	Direct labelling

Dual Colour Painting	
1597-KD-50	Biotin-Cy3 & FITC
1090-KD-50	Biotin-Texas Red & FITC
1084-KF-50	FITC & Cy3

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