# RainbowFISH™ Protocol

**Human CA-1600 4 vials 6 chromosomes each**
**Mouse CA-1601 3 vials 7 chromosomes each**

## 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 are 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.

| Kit Contents | | |
|---|---|---|
| *Product Code* | *Description* | *Volume* |
| CA-1600/1 | Probe:<br>4 vials human (Chromosomes 1-6, 7-12, 13-18 and 19-Y)<br>3 vials mouse (Chromosomes 1-7, 8-14 and 15-Y) | 5 tests |
| 1124-F1-50 | Detect F1 | 20µl |
| 1124-F2-50 | Detect F2 | 35µl |
| 1124-DT-50 | Detergent (Tween 20) | 1ml |
| 1124-Y2-50 | Detect Y2 (Cy5 Streptavidin) | 20µl |

## 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 | Deionised distilled water |
| Vortex | Fixogum rubber cement |
| Parafilm | Clear nail varnish |
| Micro-centrifuge | DAPI II® |
| 65°C, 45°C Water bath | |
| 37°C Incubator | |
| Fluorescence microscope with a suitable filter set | |

**Approx time:**

Slide preparation 90 min
Denaturation and hybridisation: 30 min + 1 day

# RainbowFISH™ Protocol

## 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:**
500µl Stock pepsin solution (1% in water)
49.5ml 10mM HCl
50ml Pepsin solution
*Pepsin stock solution can be stored at –20°C in small aliquots.*

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

**Procedure:**

**Slide preparation and denaturation:**

1. Prepare 4ml of fresh 3:1 fixative (methanol:glacial acetic acid).
2. Place tube of metaphase solution on ice.
3. Use pre-cleaned slides stored in 100% ethanol, wipe off ethanol on the surface with tissue paper and air-dry. Place on a horizontal surface.
4. Resuspend the cells in the fixative by flicking tube gently. Place a single drop of cell suspension on a slide. Check the spreading of chromosomes under phase contrast microscope. If under-spread, add a drop of fresh fixative immediately after cell suspension is placed on slide. Leave slide to dry at room temperature.

**Note**: *If cytoplasm is still present on the slides, treat the slides with pepsin (see optional pepsin pre-treatment).*

5. 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.

**Pepsin pre-treatment (optional):**

Slides can be pepsin treated on the previous day.
Dehydrate slide in 100% ethanol for 5 min and dry at room temperature.
Incubate slide in pepsin solution for 2-5 min.
(*The incubation time will vary depending on amount of cytoplasm present*).
Wash in 50ml 2XSSC for 5 min. Repeat and then rinse briefly in distilled water.
Dehydrate by serial ethanol washing for 2 min each in 70% (v/v) ethanol, 70%, 90%, 90%, and 5 min in 100%. Dry at 65°C for 1 hour and continue with Step 7 of the protocol.

6. Age for 60 min on 65°C hot plate. At this stage prepare and denature probe.

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

7. Denature slide by incubating in pre-warmed Denaturation solution at 65°C for 1½ -2 min.

8. 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.*

**Probe preparation and denaturation:**

9. Warm probe to 37°C, vortex and centrifuge for 1-3 seconds. Aliquot 10µl probe into a 500µl eppendorf tube.

10. Denature at 65°C-72°C for 10 min and then allow the probes to pre-anneal at 37°C for 10-60 min. The denaturation of probe can either be performed in a water bath, or, ideally, on a PCR machine.

**Hybridisation:**

11. Apply probe (10µl) onto the slide. Apply coverslip and remove air bubbles by gently pushing on coverslip with a pencil. Seal with rubber cement.

12. Place slide in an air tight, pre-warmed humidified chamber and incubate in the dark at 37°C overnight.

## Washing and Detection – Day Two

**Solutions to be prepared:**

1XSSC
4XSSC
Detergent wash solution
Stringency wash solution
Diluted Detection reagents

**Solution 1XSSC:**

| | |
|---|---|
| 25ml | 20XSSC |
| 475ml | Deionised distilled water |
| 500ml | 1XSSC. |

**Solution 4XSSC:**

| | |
|---|---|
| 100ml | 20XSSC |
| 400ml | Deionised distilled water |
| 500ml | 4XSSC. |

**Detergent wash solution:**

| | |
|---|---|
| 500ml | 4XSSC |
| 250µl | Detergent (Tween 20) |
| 500ml | Detergent wash solution. |

**Stringency wash solution:**

| | |
|---|---|
| 50ml | Formamide |
| 50ml | 1XSSC |
| 100ml | Stringency wash solution. |

*Stringency wash solution can be reused 5 times but should be discarded after 2 months.*

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

**Diluted Detection Reagents (DDR):**

**DDR Y2 (Cy5) + F1 (FITC)**

| | |
|---|---|
| 1.20µl | Y2 |
| 3.00µl | F1 |
| 620.8µl | Detergent Wash Solution |
| 625µl | Diluted Detection Reagent (Y2 & F1) |

**DDR F2 (FITC)**

| | |
|---|---|
| 3.0µl | F2 |
| 622.0µl | Detergent Wash Solution |
| 625µl | Diluted Detection Reagent (F2) |

**Note:** *All volumes of these working solutions are for five slides.*

# RainbowFISH™ Protocol

**Procedure:**

**Washing:**

1. Pre-warm to 45°C in a water bath at least 30 min before starting:
   Two Coplin jars of Stringency wash solution (50ml each)
   Three Coplin jars of Solution 1XSSC (50ml each)
   One Coplin jar of Detergent wash solution (50ml)

**Note:** *The temperature is important. Check the temperature of the solutions in the Coplin jar and not of the water in the water bath.*

2. Take out the slide from the incubator and carefully remove rubber cement. Place in solution 1XSSC to remove the coverslip.

3. Stringency washes:
   Wash slides twice by incubating 5 min each in Stringency wash solution (45°C).
   Wash slides twice by incubating 5 min each in Solution 1XSSC (45°C).
   Incubate slide for 4 min in Detergent wash solution. (45°C).

   Apply 125µl of pre-mixed Detection Reagent Y2 and F1 onto the slide and cover with Parafilm immediately.

4. Incubate slide in a humidified chamber for 15-20min at 37°C.

5. Remove Parafilm from the slide and wash 3 times 4 min each in Detergent wash solution at room temperature by emptying and refilling the Coplin jar.

6. Apply 125µl Diluted Detection Reagent F2. Cover with parafilm immediately and incubate at 37°C for 15-20 min.

7. Remove Parafilm from the slide and wash 3 times 4 min each in Detergent wash solution at room temperature by emptying and refilling the Coplin jar.

8. Drain slide well and mount with 50µl of DAPI II®.

9. Apply glass coverslip and seal with nail varnish. Store slides in the dark at 4°C.

10. View slides using epifluorescence filters specific for Cy3, Cy5, FITC and DAPI II®.

**Note:** *Capture pictures in the following order: Cy3, Cy5, FITC and DAPI II® and visualise.*

| Human CA-1600 | | | | |
|---|---|---|---|---|
| | Human Chromosome | FITC | Cy3 | Biotin |
| Pool I | 1 | | © | |
| | 2 | © | | |
| | 3 | | © | © |
| | 4 | © | © | |
| | 5 | | | © |
| | 6 | © | | © |
| Pool II | 7 | | © | © |
| | 8 | | | © |
| | 9 | © | © | |
| | 10 | | © | |
| | 11 | © | | © |
| | 12 | © | | |
| Pool III | 13 | © | © | |
| | 14 | | | © |
| | 15 | © | | |
| | 16 | | © | |
| | 17 | | © | © |
| | 18 | © | | © |
| Pool IV | 19 | © | | |
| | 20 | © | © | |
| | 21 | © | | © |
| | 22 | | © | © |
| | X | | | © |
| | Y | | © | |

| Mouse CA-1601 | | | | |
|---|---|---|---|---|
| | Mouse Chromosome | FITC | Cy3 | Biotin |
| Pool I | 1 | | | © |
| | 2 | © | | © |
| | 3 | | © | |
| | 4 | © | © | © |
| | 5 | © | © | |
| | 6 | | © | © |
| | 7 | © | | |
| Pool II | 8 | | © | © |
| | 9 | © | © | |
| | 10 | © | © | © |
| | 11 | © | | |
| | 12 | © | | © |
| | 13 | | | © |
| | 14 | | © | |
| Pool III | 15 | © | © | © |
| | 16 | © | © | |
| | 17 | © | | © |
| | 18 | © | | |
| | 19 | | © | |
| | X | | | © |
| | Y | | © | © |