Cy3 labelled chromosome Detection Protocol

(Cy3-09)

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 paint 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.

Kit Contents		
Product Code	*Description*	*Volume*
No Kit Required		

Requirements (not provided)

Equipment	Reagents
Ethanol cleaned slides	Sodium Chloride
Coverslips	Sodium Citrate
Eppendorf tubes Coplin jars	HCl Formamide
Humidified chamber	Absolute Ethanol
Micro-pipette 1µl, 10µl, 500µl	Fixogum rubber cement
Pipette 10ml, 20ml Vortex	Clear nail varnish Tween-20 (Available from Cambio #1124-DT-50)
Parafilm	Antifade with DAPI (Available from Cambio #1124-MD-50)
Micro-centrifuge	
45°C Water bath	
Fluorescence microscope with a suitable filter set	

Approx time:

Preparation 20 min
Procedure 40 min

Cy3 labelled chromosome Detection Protocol

(Cy3-09)

Solutions to be prepared: 20XSSC
2XSSC
Washing solution
Stringency wash solution

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 1XSSC:
25ml 20XSSC
475ml Deionised distilled water
500ml 2XSSC.

Solution 4XSSC:
100ml 20XSSC
400ml Deionised distilled water
500ml 2XSSC.

Washing solution:
250µl Tween-20
500ml 4XSSC
500ml Denaturation solution.

Stringency wash solution:
50ml Formamide
50ml 1XSSC
100ml Stringency wash solution

Stringency wash solution can be reused up to 5 times, but should be discarded after 2 months

Note: *Ensure all solutions are mixed well.*

All solution volumes sufficient for 10 slides

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 leave in Solution 1XSSC for 5 min. Take off rubber cement and replace in Solution 1XSSC to remove the coverslip.

Note: *Do not allow to dry.*

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),

Procedure: Mounting

4. Drain slide well and mount with 50µl of Antifade with DAPI.

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

Note: *You get almost no air bubbles when Cambio Antifade with DAPI (1024-MD-50) is applied on the coverslip and the almost dry (but not dried out!) slide is laid face-down on the coverslip.*

6. View slides using standard epifluorescence filters for Cy3 and for counterstain DAPI.