Biotin labelled chromosome detect with FITC Detection Protocol

(1089-KB-50)

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.

Kit Contents		
Product Code	*Description*	*Volume*
1124-B1-50	Detect B1 (FITC avidin)	30µl
1124-B2-50	Detect B2 (FITC goat anti avidin)	30µl
1124-DT-25	Detergent (Tween 20)	2 x 1ml
1124-MD-50	Reagent MD (Antifade + DAPI)	1.25ml

Requirements (not provided)

Equipment	Reagents
Ethanol cleaned slides	Sodium Chloride
Coverslips	Sodium Citrate
Eppendorf tubes	HCl
Coplin jars	Formamide
Humidified chamber	Absolute Ethanol
Micro-pipette 1µl, 10µl, 500µl	Fixogum rubber cement
Pipette 10ml, 20ml	Clear nail varnish
Vortex	Deionised Distilled water
Parafilm	
Micro-centrifuge	
45°C Water bath	
37°C Incubator	
Fluorescence microscope with a suitable filter set	

Approx time:

Preparation 20 min
Procedure 40 min

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Solutions to be prepared:
20XSSC
1XSSC
4XSSC
Detergent wash solution
Stringency wash solution
Working Reagent A
Working Reagent B
Working Reagent C

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 1XSSC

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

Detergent wash solution:
500ml 4XSSC
250µl Detergent DT
500ml Detergent wash 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

Working Reagent A:
5µl Detection reagent B1
615µl Detergent wash solution
620µl Working Reagent A (B1) (1:250)
Incubate in the dark for 5 min. Microcentrifuge at 11.000g for 5 min.

Working Reagent B:
5µl Detection reagent B2
615µl Detergent wash solution
620µl Working Reagent A (B2) (1:125)
Incubate in the dark for 5 min. Microcentrifuge at 11.000g for 5 min.

Working Reagent C: Mix supernatant of Working Reagent A and Working Reagent B.

Note: *Ensure all solutions are mixed well.*

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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 Icarefully remove rubber cement. Place 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: Detection

4. Apply 100µl of Working Reagent C onto the slide and cover with Parafilm immediately.
5. Incubate slide in a humidified box for 15-20 min at 37°C.
6. Remove Parafilm from the slide and wash 3 times for 4 min each time in the Detergent wash solution at room temperature, by emptying and refilling the Coplin jar.
7. Drain slide well and mount with 50µl of Reagent MD.
8. Apply glass coverslip and seal with nail varnish. Store slides in the dark at 4°C.

Note: *You get almost no air bubbles when supplied Reagent MD is applied on the coverslip and the almost dry (but not dried out!) slide is laid face-down on the coverslip.*

9. View slides using standard epifluorescence filters for FITC and for counterstain DAPI.