MulticolourFISH™ DEAC Protocol

Human 1813-HMF
Mouse 1803-MMF

Introduction

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

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

Kit Contents		
Product Code	*Description*	*Volume*
*delete as appropriate *1813-HMF *1803-MMF	Probe: Human Mouse	5 tests
1124-DT-50	Detergent (Tween 20)	1ml

Requirements (not provided)

Equipment	Reagents
Ethanol cleaned slides	Sodium Chloride
Coverslips	Sodium Citrate
Eppendorf tubes	HCl
Coplin jars	Pepsin
Humidified chamber	Deionised Formamide
Micro-pipette 1µl, 10µl, 500µl	Absolute Ethanol
Pipette 10ml, 20ml	Fixogum rubber cement
Vortex	Clear nail varnish
Parafilm	DAPI II®
Micro-centrifuge	
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 + 3 days

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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:
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.*

All solution volumes sufficient for 5 slides

Pepsin pre-treatment (optional):

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 5 min 100%. Dry at room temperature overnight.

Note: *Pepsin treatment replaces step 3 to 5 of Slide preparation and denaturation.*

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Procedure: Probe preparation & denaturation:

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

Note: *Use 10µl per test.*

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

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 5 min 100%. Dry at room temperature.

5. Age for 60 min on 65°C hot plate.

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.*

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

7. 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.*

Procedure: Hybridisation:

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

9. Place slide in an air tight, prewarmed humidified chamber and incubate in the dark at 37°C for 36-48 hrs.

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Washing and Detection – Day Two

Solutions to be prepared:

1XSSC
4XSSC
Detergent wash solution
Stringency wash solution

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	Deionised 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.*

All solution volumes sufficient for 5 slides

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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 remove carefully the rubber cement and place slide in Solution 1XSSC to remove the coverslip.

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

Note: *Do not allow the slide to dry.*

Procedure: Mounting:

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

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

Note: *You get almost no air bubbles when DAPI II® is applied on the coverslip and the almost dry (but not dried out!) slide is laid down on the coverslip.*

6. View slides using specific epifluorescence filters specific for *Cy5, Cy3.5, Cy3, FITC, DEAC* and DAPI II.

Note: *Capture pictures in the following order: Cy5, Cy3.5, Cy3, FITC, DEAC and DAPI II and visualize using MFISH classification software.*

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Human					
Human Chromosome	FITC	Cy3	Cy3.5	Cy5	DEAC
1	@	@		@	
2					@
3		@	@		@
4		@		@	
5	@		@		@
6	@			@	@
7	@	@			
8	@		@	@	
9			@	@	@
10		@			@
11		@	@	@	
12	@				@
13			@	@	
14	@				
15	@	@	@		
16	@			@	
17		@			
18				@	
19		@	@		
20			@		
21				@	@
22	@		@		
X			@		@
Y		@		@	@

Mouse					
Mouse Chromosome	FITC	Cy3	Cy3.5	Cy5	DEAC
1	@		@	@	
2					@
3		@		@	@
4	@		@		
5			@		
6	@			@	@
7			@	@	
8	@	@		@	
9	@	@			@
10		@			
11	@	@	@		
12				@	
13	@	@			
14				@	@
15		@	@	@	
16	@			@	
17	@		@		@
18			@		@
19		@	@		
X		@			@
Y	@				@

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