MulticolourFISH™ Classic Protocol

Human 1513-MFK
Mouse 1703-MMF

Introduction

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

On day one, the DNA of the chromosomes and paints are denatured and the hybridisation process (reannealing) takes place overnight. 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 *1513-MFK *1703-MMF	Probe: Human Mouse	5 tests
*1513-MKF-D *1703-MMF-D	Human MDA Detection Mouse MDA Detection	70µl
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	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 + 2 days

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 4ºC).

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 –20ºC 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 37ºC, vortex and centrifuge for 1-3 seconds.

Note: *Use 10µl per test.*

2. Denature probe for 10 min at 65ºC, and hold at 37ºC 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 65ºC 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 65ºC hot plate.*

6. Denature slide by incubating in pre-warmed Denaturation solution at 65ºC 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 37ºC 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
Working Reagent A

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.*

Working Reagent A:

70µl	Detection Reagent MDA
630µl	Detergent wash solution.
700µl	Working Reagent A (MDA) (1:10)

All solution volumes sufficient for 5 slides

Procedure: Washing:

1. Pre-warm to 45ºC 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.

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

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

4. Apply 125µl of Working Reagent A onto the slide and cover with Parafilm immediately.

5. Incubate slide in a humidified chamber for 15-20min at 37ºC.

6. 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.

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

8. Apply glass coverslip and seal with nail varnish. Store slides in the dark at 4ºC.

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.*

9. View slides using specific epifluorescence filters specific for Cy3, Cy3.5, Cy5, Cy5.5, FITC and DAPI II®.

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

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Human					
Human Chromosome	FITC	Cy3	Cy3.5	Cy5	Cy5.5
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	Cy5.5
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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