Bovine 1,29 Translocation FISH Protocol
(1- FITC labelled / 29 - Biotin labelled)

(CA-1624)

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.

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	Acetic Acid
Vortex	Double Distilled Water
Parafilm	Fixogum Rubber Cement
Micro-centrifuge	
45°C, 65°C Water bath	
37°C Incubator	

Approx time:

Slide preparation 30 min + overnight or + 60 min
Denaturation and hybridisation: 30 min + overnight

Note: *Metaphase spreads on slides can be prepared on previous day and aged overnight at room temperature, or on day one and aged for 60 min on hot plate.*

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

Note: *Ensure all solutions are mixed well.*

All solution volumes sufficient for 10 slides

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Procedure: Probe preparation & denaturation

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

Note: *Use a total of 15µl for a 34x22 slide, 10µl for a 22x22 slide*

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

Pepsin treatment (optional):

Note: *Pepsin treatment replaces steps 3 and 4 of Slide Preparation and Denaturation.*

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 5min 100%. Dry at room temperature.

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 5min 100%. Age for 60 min at 65°C.

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.*

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

6. 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

7. Apply probe (15µl*) onto the slide. Apply coverslip and remove air bubbles by gently pushing on coverslip with a pencil. Seal with rubber cement.

8. Place slide in an air tight, prewarmed humidified chamber and incubate overnight in the dark at 37ºC.

Note: *With good quality metaphase spreads a minimum period of 4 hours can be sufficient for hybridisation. * see note of step 1.*

Proceed with detection protocol

Note: *Post hybridisation washes are incorporated in the Detection Protocol as part of Day 2.*

Cambio recommend the use of their Detection and Amplification kits to get the best results possible with these products. Copies of all of our protocols are available on our website www.cambio.co.uk