Fluorescence *IN SITU* Hybridisation Protocol in Paraffin-Embedded Tissues Sections

Requirements (not provided)

Reagents
Phosphate Buffered Saline concentrate tablets
Sodium Thiocyanate
Tween 20
Deionised Formamide
Pepsin
Glycine
Paraformaldehyde
Standard Saline Citrate (SSC) Stock Solution
Xylene

Solutions to be prepared:

PBS
Graded Alcohols
Sodium thiocyanate Solution Pepsin solution
Glycine
Paraformldehyde Solution
Formamide Wash Solution
Stringency Wash Detergent Wash

PBS: Prepare at single and double concentration

Graded Alcohols: Use Analar grade 'absolute' ethanol and purified water to prepare 95%, 80% 69% and 30% alcohols.

Sodium Thiocyanate: Dissolve 16 g sodium thiocyanate in 200 ml purified water

Pepsin solution: Dissolve 0.8 g pepsin in 200ml of 0.1 M HCl just before use.

Glycine solution: Dissolve 0.4 g glycine in 200 ml double concentration PBS

Paraformaldehyde solution: Dissolve 8 g of paraformaldehyde (care!) in 200ml of PBS at 80 °C, cool to room temp before use. Use on day of preparation.

Formamide wash solution: 50 ml Deionised formamide mixed with 50 ml 2X SSC

Stringency wash solution: 2X SSC. Diluted from stock SSC

Detergent wash solution: Add 0.1 ml of 10% Tween-20 to 200 ml 4XSSC.

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Procedure: Probe preparation & denaturation

1. Collect 3-6 micron tissue sections on coated slides (such as Fisher Superfrost). Dry overnight at 37°C
2. Dewax in Xylene, 3 x 5 min each
3. Rehydrate through graded alcohols (95%, 80% 69% and 30%) to water
4. Incubate with sodium thiocyanate solution for 10 mins at 80°C (Care!)
5. Wash in PBS
6. Incubate in Pepsin solution for 10 min at 37°C (see notes)
7. Quench the pepsin in Glycine solution
8. Wash in PBS
9. Post-fix in paraformaldehyde solution for 2 min
10. Wash well in PBS: 3 changes over 15 min
11. Dehydrate through graded alcohols then air dry
12. Remove the pre-diluted Chromosome Paint from the freezer, mix well and warm to 37°C
13. Apply 10-15µl paint mix to the centre of the slide
14. Cover with a glass coverslip (22X40mm) and seal with Fixogum Rubber Cement
15. Denature the sealed slide at 100 °C in a boiling water bath for 10 min
16. Place the slide horizontally in a humid chamber and hybridise overnight at 37°C
17. Carefully peel of the rubber cement an d remove the coverslip
18. Wash in Formamide Wash Solution at 37°C for 3 changes of 5 min each
19. Wash with Stringency Wash solution at 37°C for 3 changes over 15 min
20. Wash with Detergent Wash Solution at 37°C for 10 min
21. Wash with PBS, 3 changes over 15 min
22. Mount in reagent MD (DAPI & Mountant) and examine

Notes:

Many of the reagents and solutions require specific precautions: read the products inserts datasheets.

Digestion times for Pepsin solution need to be determined for individual tissues. This will depend upon the type and length of fixation as well as the tissue type itself.

The Sodium thiocyanate step appears to be crucial. We recommend that this step is kept constant, but explore different Pepsin digestion times. Pepsin is known to autodigest. We recommend that pepsin powder is added to 0.1 M HCl whilst slides are washing in PBS after step 4.

It is possible to carry out immunohistochemistry before chromosome detection, and to use indirect methods to visualise the chromosome signals [See Poulsom et al. (2001). The Journal of Pathology. 195: 229-235].

This method was prepared by Rosemary Jeffery and Richard Poulsom of the Histopathology Unit, Cancer Research UK, London.