In Situ Hybridisation Method

Please read the procedural notes before commencing this protocol.

Sample Preparation

1. Dewax slides in xylene for 2 x 3 minutes.
2. Hydrate in 99% v/v ethanol for 2 x 3 minutes.
3. Hydrate in 95% v/v ethanol for 3 minutes.
4. Immerse in water for 3 minutes (see Procedural Notes, point 4).
5. Place slides on an incubation tray and cover with 100µl of proteinase K in 0.05M Tris/HCl buffer pH 7.6 and incubate for 30 minutes at 37°C.
6. Immerse in water for 2 x 3 minutes (see Procedural Notes, point 4).
7. Dehydrate in 95% v/v ethanol for 3 minutes.
8. Dehydrate in 99% v/v ethanol for 3 minutes.
9. Air dry.

Hybridisation

1. Add 20µl of probe hybridisation solution to slides as required and coverslip sections.
2. Incubate for 2 hours at 37°C (see Procedural Notes, point 6).
3. Allow coverslips to drain off into a beaker.
4. Wash slides in TBS containing 0.1% v/v Triton X-100 for 3 x 3 minutes.

Detection

Reagents supplied in the Novocastra *In Situ* Hybridisation Detection Kit (NCL-ISH-D) can be used in steps 2 and 5 of the following method. When the kit is used, please refer to the supplied instructions for use of the reagents.

1. Place slides on incubation tray and cover sections with 100µl of normal rabbit serum diluted 1:5 in TBS containing 3% w/v BSA, 0.1% v/v Triton X-100. Incubate for 10 minutes.
2. Tip off the blocking solution and add Rabbit F(ab') anti-FITC conjugated to alkaline phosphatase diluted 1:200 in TBS containing 3% w/v BSA and 0.1% v/v Triton X-100. Incubate for 30 minutes.
3. Wash slides in TBS for 2 x 3 minutes.
4. Wash slides in alkaline phosphatase substrate buffer pH 9.0 for 5 minutes.
5. Place slides in incubation tray and demonstrate alkaline phosphatase activity by covering the sections with 100µl of the following solution:

10ml	Alkaline phosphatase substrate buffer
80µl	5-bromo-4-chloro-3-indolyl phosphate (BCIP) (50mg/ml in dimethyl formamide)
80µl	Nitro blue tetrazolium (NBT) (75mg/ml in 70% v/v dimethyl formamide)
10µl	1M levamisole

Coverslip and incubate at room temperature in the dark overnight.

6. Wash in running water for 5 minutes.
7. Counterstain in Mayer's haematoxylin for a maximum of 10 seconds.

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8. Mount in aqueous mountant.

Suggested Solutions

TBS: 50mM Tris/HCl, 150mM NaCl pH 7.6

Alkaline phosphatase substrate buffer: 100mM Tris/HCl, 50mM $MgCl_2$, 100mM NaCl pH 9.0.

Procedural Notes

1. Use of the control probe (NCL-CONTROL) is recommended alongside the application of specific probes to control for non-specific probe interactions with tissue. In the small and large intestine, cells of the diffuse endocrine system will react non-specifically with the probes and in this situation the control probe should always be applied to duplicate sections.
2. For surgical tissues fixed in formalin for 24-48 hours and paraffin wax-embedded, digestion with a range of proteinase K concentrations of 5, 10 and 15µg/ml should be carried out. Under and over digestion will result in suboptimal strength of signal.
3. The probe hybridisation solution contains formamide and care should be taken to avoid skin contact.
4. All solutions should be prepared using high grade chemicals and highest quality water available in the laboratory, ie, reverse osmosis and deionised or double distilled.
5. For tissues fixed in solutions containing mercury, it is necessary to pretreat sections before the proteinase K step with 0.2M HCl for 20 minutes, then wash in water for 2 x 5 minutes. This procedure is essential to reduce background staining resulting from mercury fixation.
6. Endogenous alkaline phosphatase activity can be blocked by denaturing sections prior to hybridisation. For denaturation, the preparations should be covered with the probe solution and coverslipped as for the standard procedure. The preparation should then be heated at 65°C for 15 minutes, after which hybridisation at 37°C should proceed for the standard time of 2 hours.