DIG-LABEL IN SITU HYBRIDIZATION

Tissue Preparation: Using fresh frozen tissue will give the maximum signal but histology quality may be sacrificed. An overnight fixation would be optimal for obtaining very good sensitivity while preserving histology. (When testing fixation times and sensitivity, fresh frozen was the most sensitive, overnight fixed tissue was the second most sensitive. Fixation times less than o/n were not as sensitive. However, when combining in situ with immunohistology, shorter fixation times might be necessary to detect protein.

Day1

1. Section embryos using superfrost slides (fresh frozen or over night fixed tissue is best).
2. Air dry
3.

12. Pour off hyb buffer from slide, dab off the edge with kimwipe to remove excess hyb buffer and put 100ul of probe in hyb buffer per slide.
13.

29. Coverslip with DAKO Cytomation Glycergel Mounting Medium (C0563) at 60oC
30. Indulge in results.

MAKING DIG LABELED PROBE

Either:

1. Linearize 10ug of plasmid (T7, T3, or SP6 promoter)
2. Purify with gel purification columns or Extract with Phenol /Chlorform /Isoamylalcohol and ppt with 2.5 volumes EtOH and 1/10 Vol sodium Acetate

Or : Preferred method

1. PCR your gene of interest including a 3' promoter sequence (T7, T3, or SP6) at the 3' end.
2. Gel purify your fragment.
3. Resuspend in Rnase free EB
4. For Transcription, follow the protocol provided in the kit (Roche cat # 1-175 025).
5. Remove unincorporated NTPS by passing through G50 column.
6. Resuspend the probe (50ul) in an additional 100ul Hyb buffer to prevent degradation and freeze/ thaw.
7. Store at -20oC.

SOLUTIONS

0.2m Phosphate Buffer (PB) pH7.2 4 L

165.3 g Na2HPO4.7H2O (MW268.07)
25.6 g NsH2PO4.H2O (MW 137.99)
Water to 4L

4% Paraformaldehyde in PBS (400 ml)

200ml H2O
10ul 10N NaOH
16 g paraformaldehyde powder (PFA)
200 ml 0.2M PB pH7.2
3 g NaCl

Heat water to 70oC on hot/stir plate and add PFA.
Stir with magnetic stir bar to even suspension.
Add 10ul NaOH. When suspension clears add 0.2MPB and 3 g of NaCl.
Filter and chill on ice.

PBS (0.1MPB 0.15MNaCl) pH7.2 4L

2L 0.2M PB pH7.2
35 g NaCl
Water to 4L

Proteinase K buffer (ProtK 1ug/ml in 50mM TrisHCl pH7.5 and 5mM EDTA 400 ml

5 ml m [(W)F1 1 q BTCC

Roche (11681 451 001)
For 10 ml

200ul stock solution
0.24 mg/ml Levamisole (optional)
0.1% Tween 20 (100ul of 10%)
10 ml Buffer B3

Vector (SK5400)
For 5 ml

2 drops of NBT
2 drops of BCIP
1 drop Levamisole (optional)
0.1% Tween 20 final (50ul of 10%)
5 ml B3