In situs on paraffin sections (modified Paris protocol)

Prehyb:

Dewax slides in histosol 2 x 5 min
100% EtOH 2 x 5 min, then 2 min each of 90%, 70%, 50% EtOH/DEPC PBS, DEPC water, DEPC PBS + 0.1% Tween. Rinse in 2x SSC.

Hyb:

Dilute digUTP probe in hyb mix (optimal concentration to be determined empirically, but 1ng/uL typically works well). Prepare 300µl of probe + hyb, and apply 250µl of probe + hyb per slide. Incubate under glasscoverslip at 65-70°C overnight in 50% formamide, 2xSSC-humidified chamber (e.g. plastic container with Pasteur pipette rungs).

Washes:

Wash 2 x 30 min in pre-warmed 50% formamide, 1x SSC, 0.1% Tween-20 at 65-70°C (in coplin jars). Coverslips should fall off during first wash.

Wash 3 x 10 min in MABT at room temp.

Anti-DIG antibody staining:

Block for 2 hours at room temp with 1% Roche blocking reagent and 20% sheep serum (in a LockMailer slide jar –71406-10 from https://www.emsdiasum.com/microscopy/products/histology/mailers.aspx).

Dilute Boehringer anti-DIG-AP 1/2000 in above solution, and apply overnight in LockMailer slide jar at room temperature.

Next day: Rinse 5 x 30 min in MABT in agitated bath, then frequent rinses through the day.

Next day: equilibrate 3 x 10 min in NTMT

Colour reaction

Reveal color using BM Purple ready-to-use AP substrate.

Once complete, wash in PBS; post-fix 20' RT in 4% paraformaldehyde, rinse in PBS and coverslip with aqueous mounting medium (e.g. fluoromount G).

Solutions

10x salt solution (pH 7.5):

NaCl	23.38g	(2M)
Tris HCl	2.808g	(=0.089M, equiv. 2.16g Tris base)
Tris base	0.265g	(=0.011M i.e. **total Tris=0.1M, pH7.5**)
$NaH_2PO_4.H_20$	1.38g	(0.05M)
Na_2HPO_4	1.42g	(0.05M i.e. **total PO_4 = 0.1M**)
0.5M EDTA	20 ml	(0.05M)

make up to 200ml with DEPC-treated water --- this is enough to furnish the whole lab and it lasts forever.

Hyb mix:	*final conc.*	*volume*
10x salt	1x	1 ml
formamide	50%	5 ml
50% dextran sulfate stock	10%	2 ml
yeast tRNA 10 mg/ml stock	1 mg/ml	1 ml
50x Denhardt's	1x	200 µl
DEPC-treated water		to 10ml (800µl)

Making 50ml hyb mix

Dissolve 5g dextran sulfate (Sigma D-8906) with 10 ml DEPC-treated H_2O in a 50ml Falcon tube (place the tube on a shaking platform). This might take 2-3 hours. It will turn very viscous. Make sure all dextran sulfate is dissolved before adding 10x salt solution, yeast RNA and 50x Denhardt's solution. Make up to 25ml with DEPC-H_2O. Add 25ml deionised formamide and return the tube to the shaking platform for another 30 minutes or until all ingredients are properly mixed. Aliquot into 5ml and 10 ml and store at –20°C.

20x SSC

NaCl	175.3 g
Na-Citrate ($2H_2O$)	88.2 g
Water	to 1 litre

Adjust to pH 5.0 with citric acid. Treat with DEPC (1:1000) overnight and then autoclave.

10x MAB (pH 7.5)

Maleic acid	116 g
NaCl	87 g
Water	to 1 litre

Dissolve Maleic acid in 700ml H_2O adjust to pH 6.5 with NaOH pellets (you'll need quite a lot of NaOH pellets- about 75g. Keep stirring during the addition of NaOH. To avoid excess heat causing the solution boiling, add NaOH in small batches. The solution will start around pH1.8 and looks clear. As the pH goes up, it will turn cloudy, until about pH5.5, when it turns clear again. Make sure the pH is measured when all the pellets are dissolved and the solution has cooled to RT). Then pH with Tris base (about 15g) to pH7.5. Once the pH is done, add NaCl and H_2O making up to 1L.
The solution is autoclavable for long-term storage.

NTMT:

5M NaCl	2ml	(0.1M)
1M Tris pH 9.5	10ml	(0.1M)
1M $MgCl_2$*	5ml	(5mM)
Tween-20	100µl	(0.1%)
water	to 100 ml	

* Caution: when making $MgCl_2$ stock solution: ALWAYS add $MgCl_2$ solid into H_2O.