

Pre-treatment of cryosections for mRNA detection in tissue sections^a

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Reagents

- ◆ PFA: freshly prepared as described in [Fixation and storage of mouse embryos for mRNA detection](#)
- ◆ Solution 1: 50% formamide, 2 × SSC

Method

Cryosections are useful for very late stage embryos and adult tissue sections. For embryos up to late stage gestions see [Wax embedding of mouse embryos for mRNA detection](#).

- 1 Place slides in cold acetone (4 °C) for 5 min and then air dry.
- 2 Fix in PFA for 15 min at 4 °C.
- 3 Wash slides twice in PBS for 5 min.
- 4 Acetylate exactly as described in *Pre-treatment of sections*, step 11. 'Warning'
- 5 Wash twice in 2 × SSC for 5 min at room temperature.
- 6 Place slides in pre-warmed solution 1 for 10 min at 60 °C.
- 7 Quickly transfer slides to pre-cooled 50% then 70% ethanol at -20 °C for 5 min.
- 8 Dehydrate slides in 100% ethanol twice (5 min each) at room temperature and air dry.
- 9 Hybridization, washing, and autoradiography are carried out exactly as described in [Hybridization of mouse embryo sections for mRNA detection](#), [Post-hybridization washes after mRNA detection in mouse embryo tissue sections](#), [Autoradiography for mRNA detection in mouse embryo tissue sections](#)^a, and [Developing and staining slides for mRNA detection in mouse embryo tissue sections](#).