

Pre-treatment of sections before mRNA detection

Antonio Simeone

MRC Centre for Developmental Neurobiology, New Hunt's House, King's College London, Guy's Campus, London Bridge, London SE1 1UL UK.
International Institute of Genetics and Biophysics, CNR, Via G. Marconi, 12, 80125 Naples, Italy.

Reagents

- ◆ PFA, PBS, saline (see [Fixation and storage of mouse embryos for mRNA detection](#))
- ◆ Dilution buffer for proteinase K: 50 mM Tris-HCl, 5 mM EDTA pH 8—sterilize by autoclaving
- ◆ 0.1 M triethanolamine-HCl pH 8—sterilize by autoclaving
- ◆ Proteinase K: freshly dilute from a 10 mg/ml stock to a final concentration of 1 µg/ml in dilution buffer
- ◆ Acetic anhydride

Acetic anhydride is toxic and must be dispensed in a fume-hood

Method

- 1 Dewax slides in xylene^d twice for 10 min.
- 2 Place slides in 100% ethanol for 2–3 min to eliminate xylene.
- 3 Wash slides in 100% ethanol (twice), and then in decreasing concentrations of 95%, 85%, 60%, and 30% ethanol for 5 min each.
- 4 Transfer slides into saline solution and then PBS for 5 min each.
- 5 Refix slides in freshly prepared PFA for 20 min.
- 6 Wash twice with PBS for 5 min.
- 7 Transfer slides to proteinase K solution and leave at 37 °C for 30 min.
- 8 Wash in PBS for 5 min.
- 9 Repeat fixation of step 5.
- 10 Wash in distilled water for 30 sec and place in 0.1 M triethanolamine-HCl in a container with a rotating stir bar placed in a fume-hood.
- 11 Add acetic anhydride to a concentration of 0.25% (v/v) and leave for 10 min. When the acetic anhydride has fully dispersed the stirrer can be turned off.
- 12 Wash with PBS and saline solution for 5 min each.

- 13 Dehydrate slides by passing through 30%, 60%, 85%, 95%, and 100% (twice) ethanol for 2–3 min each.
- 14 Air dry and use for hybridization.

Notes

- a The volume can be adjusted according to the number of slides and to the size of the container. We try to combine experiments to obtain a final number of 25 or 50 slides. For 50 slides, 400 ml for each solution are enough. Xylene or ethanol can be used several times and stored in bottles at room temperature.