

Fixation and sectioning of cryosections for mRNA detection^a

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: freshly prepared as described in [Fixation and storage of mouse embryos for mRNA detection](#)
- ◆ Sucrose solution: 18% sucrose in PBS
- ◆ Tissue-Tek OCT medium

Method

Use for very late stage embryo and adult tissues. For analysis of embryos up to late stages of gestation see [Wax embedding of mouse embryos for mRNA detection](#).

- 1 Perfuse terminally anaesthetized mice with PFA by heart injection.
- 2 Dissect out brain and place it in PFA at 4 °C for an additional 1–2 h with gentle rotation.
- 3 Wash in PBS for 30 min, then place in sucrose solution, and gently rotate at 4 °C overnight.^b
- 4 Transfer the brain to a mould containing Tissue-Tek OCT medium, orientate with a needle, and place in dry ice for 2 h.^c
- 5 Set the cutting temperature of the cryostat to –20 °C, apply the OCT cube to the cryostat, orientate, and cut 8–10 µm sections.
- 6 Transfer sections one by one from the blade to the slides.
- 7 Dry the slides in the cryostat at –20 °C for 2 h or overnight.
- 8 Fix the dried slides in PFA for 3–5 min.
- 9 Wash briefly with PBS twice.
- 10 Dehydrate by passing through 30%, 60%, 85%, 95%, and 100% (twice) ethanol, and then air dry.
- 11 Slides can be stored at –80 °C or pre-treated for hybridization (see [Pre-treatment of cryosections for mRNA detection in tissue sections](#)).

Notes

- a This method is for brain sections but has been successfully used for other adult tissues.
- b Steps 2 and 3 can be omitted and the brain directly transferred to the mould, but sucrose helps to prevent damage and gives better histological preservation of the tissue.
- c At this step the mould can be stored at $-80\text{ }^{\circ}\text{C}$.