

Fixation and storage of mouse embryos for mRNA detection

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Equipment and reagents

- ◆ Stereo dissecting microscope
- ◆ PBS: 8 g NaCl, 0.2 g KCl, 1.44 g Na₂HPO₄, 0.24 g KH₂PO₄ dissolved in 1 litre of distilled water and pH adjusted to 7.4—sterilize by autoclaving
- ◆ PFA: 4% paraformaldehyde dissolved in PBS at 55–65 °C and then cooled on ice
- ◆ Saline solution: 8.3 g of NaCl in 1 litre of distilled DEPC treated water—sterilize by autoclaving

Method

- 1 Dissect out embryos in a Petri dish under stereomicroscope in ice-cold PBS.
- 2 Transfer the embryos to a fresh Petri dish containing PBS and wash for a few minutes, shaking very slowly.
- 3 Transfer the embryos to a vial containing ice-cold freshly prepared PFA^a and leave at 4 °C for 8–16 h.^b
- 4 Replace PFA with saline solution and wash two times with gentle rotation for 30–90 min^c at 4 °C.
- 5 Wash with 1:1 saline solution:ethanol for 30–90 min^{c, d} at 4 °C.
- 6 Wash with 70% ethanol for 30–90 min^c at 4 °C.
- 7 Repeat step 6 and store at 4 °C.^d

Notes

- a All the steps from fixation to wax embedding should be performed using at least 10 ml of solution for three to eight early embryos (6.5–10.5 d), 20 ml for two to four mid-gestation embryos (12.5–14.5 d), and 20–30 ml or more for one or two embryos of later stages.
- b Younger embryos from 8.5–10.5 d without the decidua should be fixed for no more than 12–16 h; they are generally already fixed after 6–8 h. For older embryos (15–19 d), fixation should be prolonged to 48 h. In this case it is wise to replace

fixative after 20–24 h. To improve fixation of very late embryos, cut carefully into the belly and the vault of the cranium.

- c Washes are increased in length depending on the size of embryos.
- d Embryos can be stored in 70% ethanol indefinitely at 4 °C.