

Pre-treatment of sections or cells on slides

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Equipment and reagents

- ◆ Slide racks and appropriate containers (e.g. 250 ml plastic boxes or glass dishes)
- ◆ 4% paraformaldehyde in PBS (see [Fixation and pre-treatment of embryos for whole mount hybridization](#))
- ◆ PBS
- ◆ 10 µg/ml proteinase K: freshly diluted in PBS from a 10 mg/ml stock in distilled water

Method

- 1 (a) For dehydrated cryostat sections or cells on slides: warm up to room temperature. Fix in 4% paraformaldehyde in PBS for 20 min. Wash three times for 5 min each in PBS.

(b) For wax sections: dewax the slides in HistoClear, twice for 10 min. Wash in 100% methanol for 2 min to remove most of the HistoClear. Transfer the slides through 100% methanol (twice), 75%, 50%, and 25% methanol in PBS, for 1–2 min in each solution. Wash twice in PBS for 5 min each. Optional: immerse the slides in fresh 4% paraformaldehyde in PBS for 20 min, then three times for 5 min in PBS.
- 2 Drain the slides and place horizontally on a clean sheet of absorbent paper on the bench. Overlay the sections with 10 µg/ml proteinase K and leave for 5–10 min.
- 3 Shake off excess liquid and wash the slides with PBS for 5 min.
- 4 Fix in fresh 4% paraformaldehyde in PBS for 20 min.
- 5 Wash the slides three times with PBS for 5 min.
- 6 Dehydrate by passing through 25%, 50%, 75% methanol in PBS, then twice in 100% methanol, for 1–2 min in each solution.
- 7 Air dry. Should be used on the same day for hybridization (though if required, can be stored in airtight container at –20 °C for at least several days).