

## Post-hybridization washes after mRNA detection in mouse embryo tissue sections

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### Equipment and reagents

- ◆ Rack for 25 slides
- ◆ Slide jar or plastic box suitable for the slide rack
- ◆ 20 × SSC: 3 M NaCl, 0.3 M sodium citrate dihydrate
- ◆ Solution 1: 5 × SSC, 15 mM DTT—pre-warm to 55 °C just before use
- ◆ Solution 2: 50% formamide, 2 × SSC, 15 mM DTT—pre-warm to 65 °C just before use
- ◆ NTE buffer: 0.5 mM NaCl, 10 mM Tris-HCl pH 7.5, 5 mM EDTA pH 8
- ◆ RNase solution: dilute ribonuclease A in NTE buffer to a final concentration of 20 µg/ml

### Method

- 1 Remove the slide rack, place in 250 ml solution 1, then gently remove the Parafilm pieces covering the slides and discard them.
- 2 Transfer the slide rack to 250 ml solution 1 and incubate at 55 °C for 1 h.
- 3 Transfer to solution 2 at 65 °C for 1 h.
- 4 Wash three times for 15 min each with NTE buffer at room temperature.
- 5 Treat with RNase solution at 37 °C for 30 min.
- 6 Wash with NTE buffer for 15 min at room temperature.
- 7 Repeat step 3.
- 8 Wash in 2 × SSC and then in 0.1 × SSC for 15 min each at room temperature.
- 9 Dehydrate slides by passing through 30%, 60%, 85%, and 95% ethanol, all including 0.3 M ammonium acetate, followed twice by 100% ethanol.
- 10 Air dry.