# Developing and staining slides for mRNA detection in mouse embryo tissue sections

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## Reagents

- Developing solution: Kodak D19 developer at 160 g/litre
- Fixing solution: Kodak Unifix at 130 g/litre

## A. Developing slides

1 Remove box with slides from 4 °C and leave 1 h at room temperature.

2 In the dark-room transfer the slides to a slide rack.[a]

3 Place the slide rack in a freshly prepared developing solution for 2 min, in 1% glycerol:1% acetic acid (alternatively a wash in water is sufficient) for 1 min, then in fixing solution for 5–10 min, all at room temperature.

4 Wash slides twice in a large volume of water for 10 min each.

5 Place slides in 0.02% toluidine blue solution[b] for 5 min, then wash in water for 1–2 min.

6 Dehydrate and destain[c] slides by passing through 30%, 60%, 85%, 95% ethanol, then twice in 100% ethanol, and then air dry.

7 Mount the slides with a clean coverslip using a few drops of a mounting agent (Permount or DPX) and dry in a fume-hood.

## B. Rescuing slides from high background in the emulsion

**If a high level of background is detected in the emulsion upon checking one or two experimental slides, proceed as follows with the remaining slides.**

1. Transfer the undeveloped slides to a slide rack.
2. Place the slide rack in a low concentration fixing solution (30 g/litre) for 20 min; the slides will appear completely transparent.
3. Wash four times in a large volume of 0.1 × SSC for 20 min each.

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4. Dehydrate slides through 30%, 60%, 85%, 95%, then twice in 100% ethanol, and then air dry.
5. Repeat autoradiography procedure ( see Autoradiography for mRNA detection in mouse embryo tissue sectionsa) and develop again.

## Notes

a Before developing all the slides, check one or two of them to verify that there is no background.

b Alternative stains can be used such as eosin, haematoxylin/eosin, or Giemsa.

c To better visualize faint signals we suggest a pale blue stain.

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