

Whole mount hybridization, washing, and detection of probe (method 1)

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Equipment and reagents

- ◆ Shaking platform and heater block, or shaking platform in 55–67 °C incubator
- ◆ Petri dishes or multiwell culture dishes
- ◆ Sheep serum. It may be beneficial to heat inactivate the serum at 56 °C for 30 min. To avoid high backgrounds, it is important that it is free of endogenous phosphatases. Once a good batch has been identified, store in aliquots at –20 °C.
- ◆ 20 x SSC stock solution
- ◆ 10% CHAPS stock solution (store at –20 °C)
- ◆ Washing solution 1 (for zebrafish embryos): 2 x SSC, 0.01% Triton X-100
- ◆ Washing solution 1 (for other embryos): 2 x SSC, 0.1% CHAPS
- ◆ Washing solution 1 containing 25% formamide
- ◆ Washing solution 2 (for zebrafish embryos): 0.2 x SSC, 0.1% Triton X-100
- ◆ Washing solution 2 (for other embryos): 0.2 x SSC, 0.1% CHAPS
- ◆ PBT (see [Fixation and pre-treatment of embryos for whole mount hybridization](#))
- ◆ KTBT: 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 10 mM KCl, 0.1% Triton X-100
- ◆ NTMT: 100 mM Tris-HCl pH 9.5, 50 mM MgCl₂, 100 mM NaCl, 0.1% Triton X-100
- ◆ AP-conjugated anti-DIG or anti-fluorescein antibody (Boehringer)
- ◆ NBT and BCIP stock solutions (Boehringer) or BM Purple (Boehringer)
- ◆ Paraformaldehyde fixative (see [Fixation and pre-treatment of embryos for whole mount hybridization](#))

Method

- 1 Incubate embryos with hybridization mix containing probe overnight at 55–67 °C on shaking platform.
- 2 Wash in washing solution 1 containing 25% formamide, three times for 10 min, at 55–67 °C on shaking platform.
- 3 Wash in washing solution 1 for 20 min at 55–67 °C on shaking platform.

- 4 Wash with washing solution 2, three times for 20 min, at 55–67 °C on shaking platform.
- 5 Rinse with KTBT twice for 10 min each at room temperature. Alternatively use PBT in place of KTBT for this and all subsequent steps.
- 6 Pre-block the embryos with 10% sheep serum in KTBT for 1–3 h at room temperature on shaking platform.
- 7 Incubate with 1/2000 dilution of anti-DIG or anti-fluorescein antibody (optional: can be pre-absorbed with embryo powder as described in [Pre-absorption of antibody](#)) in 10% sheep serum in KTBT, and rock overnight at 4 °C. The antibody solution can be recovered, stored at 4 °C and used up to three times in total; for storage the solution must contain 0.01% sodium azide.
- 8 Wash the embryos with KTBT five or more times for 1 h each at room temperature, then overnight at 4 °C on shaking platform. The overnight wash is optional, but gives lower backgrounds (especially for larger embryos), and it is more convenient to monitor a slow colour reaction if it is started on the following morning.
- 9 Wash twice in NTMT for 15 min and transfer to a Petri dish or culture dish for easier observation. The NTMT can include 1 mM levamisol, which inhibits many endogenous alkaline phosphatases, but for many systems this is not needed.
- 10 Incubate in the dark with NTMT containing 4.5 µl/ml NBT stock solution, 3.5 µl/ml BCIP stock solution. Alternatively, use BM Purple solution; this reagent gives a stronger signal and low background but may not stain deeper tissues. Periodically monitor the reaction and when a strong signal is produced and/or any background is observed, stop by washing several times with PBT. Fix in 4% paraformaldehyde in PBS for 1–2 h at room temperature, and store in PBS at 4 °C.