

Pre-absorption of antibody

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Equipment and reagents

- ◆ Shaking platform at 4 °C
- ◆ Microcentrifuge
- ◆ Embryo powder

Embryo powder should if possible be prepared from the same species as you are carrying out *in situ* hybridization on, but does not need to be from the same developmental stage (more material can be prepared from later stage embryos)

- ◆ Sheep serum
- ◆ KTBT or MABT
- ◆ AP-conjugated anti-DIG or anti-fluorescein antibody

A. Preparation of embryo powder

- 1 Homogenize embryos in a minimum volume of PBS.
- 2 Add 4 vol. of ice-cold acetone, mix, and incubate on ice for 30 min.
- 3 Centrifuge at 10 000 *g* for 10 min and remove supernatant.
- 4 Wash pellet with ice-cold acetone and centrifuge again.
- 5 Spread the pellet out and grind it into a fine powder on a sheet of filter paper.
- 6 Air dry and store in an airtight tube at 4 °C. Is stable for years.

B. Pre-absorption of antibody

1. For 2 ml of final solution, place ~ 3 mg embryo powder in a microtube and add 0.5 ml 10% sheep serum in KTBT (if using [Whole mount hybridization, washing, and detection of probe \(method 1\)](#)) or MABT (if using [Whole mount hybridization, washing, and detection of probe \(method 2\)](#)) and 1 µl antibody.
2. Shake tube (lying on its side) gently at 4 °C for at least 2 h.
3. Spin for 1 min in a microcentrifuge, remove the supernatant, and dilute it to 2 ml in buffer. The pre-absorbed antibody is stable at 4 °C.