

Immunohistochemistry

- Embryos harvested after collection on grape juice plates and either formaldehyde/methanol or methanol fixed. Egg Chambers isolated and fixed via standard protocol.
- Rehydrate embryos (~50-100 μ l embryos/1.5ml microfuge tube) through methanol series: 90% MeOH, 75% MeOH, 50% MeOH and finally PBST. Each wash consists only of several inversions of the tube. (This step is not necessary for egg chambers.)
- Embryos should be permitted to rehydrate in PBST for 30 minutes. (Not necessary for egg chambers - start at the next step)
- Following fixation incubate the embryos/egg chambers in 1% Triton X-100 in PBS for 1 to 2 hours at room temperature (rock gently, 2 hours recommended)
- Rinse twice with PBST and continue.
- 1° Antibody: Prepare the appropriate dilution of the primary antibody in PBST (or PBST + 1% BSA). Incubate the embryos/egg chambers with the primary antibody overnight at 4° C. Constant gentle mixing is required during all incubations and washes.
- Rinse embryos/egg chambers 2 \times with PBST. Wash the embryos/egg chambers 3 \times in PBST (~15 min each) at room temperature.
- 2° Antibody: Prepare the appropriate dilution of the secondary antibody in PBST. Incubate the embryos/egg chambers with the secondary antibody overnight at 4° C for the best results (2-4 hours at room temperature is also possible). (Most 2° antibodies are used at 1:250) Note: Sometimes it is necessary to pre-absorb 2° antibodies due to non-specific binding. Pre-absorb antibodies by diluting in PBST and incubating with wild-type embryos overnight at 4° C.
- Wash the embryos/egg chambers 3 \times in PBST (~15 min./wash) at room temperature with gentle agitation.
- (Optional: DNA staining with TOTO3 or DAPI)
- Mount immunolabeled embryos/egg chambers in PBS/Glycerol + p-Phenylenediamine Dihydrochloride or clear and mount in BABB (see clearing protocol).

Solutions and Reagents

PBST

Reagent	Quantity	Concentration
10× PBS	50 ml	1×
Triton X-100 (or 10% Triton X-100)	250µl (2.5 ml 10% Triton X-100)	0.05%

- Adjust volume to 500 ml with dH₂O.

10× PBS

Reagent	Quantity	10× Concentration
NaCl	74.8 g	1.28 M
KCl	1.5 g	0.02 M
KH ₂ PO ₄ (MW136.1)	2.7 g	0.08 M
Na ₂ HPO ₄ •7H ₂ O (MW 268.07)	21.4 g	0.02 M

- pH ~7.2-7.4
- Adjust volume to 1 L with dH₂O

Mounting Medium w/1,4-Phenylenediamine dihydrochloride

Reagent	Quantity
p-Phenylenediamine Dihydrochloride	100 mg
10× PBS	10 ml
Glycerol	90 ml

- Store in aliquots at -20°C (discard when solution appears dark purple/brown)

Reagent	Vendor	Catalog #
1,4-Phenylenediamine dihydrochloride	Sigma	P1519
Triton X-100	Sigma	T9284
10% Triton X-100	Roche	1332481