

Formaldehyde/Methanol Fixation for Embryos

1. Collect embryos on grape juice agar plates.
2. Rinse the embryos from the plates with Triton/NaCl (0.05% Triton X-100, 0.7% NaCl) and collect on a fine nylon mesh (Nitex nylon membrane – 200 mesh) supported in a filter assembly. (Note: Less is better than more, large quantities of embryos can cause clumping which may produce anoxic conditions, potentially inducing anaphase bridging. To avoid anoxia, embryos should maximally form a sparse monolayer in the filter assembly.)
3. Briefly rinse collected embryos with Triton/NaCl.
4. Transfer the filter assembly with the embryos to a beaker containing a solution of 50% bleach:50% Triton/NaCl. Incubate 2-3 min to remove the chorion (swirl gently).
5. Rinse the embryos with Triton/NaCl and then with dH₂O.
6. Draw water away from the embryos by placing paper towels against the nylon mesh.
7. Place the embryos in a 25 ml (50 ml) Erlenmeyer flask containing 5 ml (10 ml) 100% n-Octane. Gently swirl the embryos in the n-Octane for 20-30 sec (actually: the time it takes to transfer the embryos to the n-octane and a quick swirl is sufficient) to permeabilize the vitelline membranes.
8. Add 5 ml (10 ml) 37% formaldehyde to the embryo/n-Octane mixture and incubate for 3-5 min (place on shaker). The embryos float at the interface between the formaldehyde (lower phase) and the n-Octane (upper phase).
9. Draw off the lower phase (formaldehyde).
10. Add 5 ml (10 ml) 100% methanol, stopper the flask, and shake vigorously for ~30 sec (removes vitelline membranes). Embryos that have lost their vitelline membranes sink to the bottom. Embryos that have not lost their vitelline membranes remain at the methanol/n-Octane interface.
11. Draw off the n-Octane, any embryos remaining at the interface, and most of the methanol.
12. Resuspend the remaining embryos in 100% methanol and incubate for 1 to 2 hours at room temperature. (Embryos may be stored in 100% methanol at –20° C at this point)
13. Prior to use the embryos must be rehydrated, passage embryos through a methanol series: 90% MeOH, 75% MeOH, 50% MeOH and PBST.
14. Incubate the embryos in PBST for 30 min at room temperature.
15. Proceed to Immunohistochemistry or in situ hybridization.

Note: Formaldehyde/Methanol fixation is ideal for visualizing astral microtubules.

Solutions and Reagents

Triton/NaCl (10×)

Reagent	Quantity	10× Concentration
Triton X-100	2.5 ml	0.5%
NaCl	35 g	7%

- Adjust volume to 500 ml with dH₂O
- Dilute to 1× working concentration with dH₂O prior to use

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

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.

Reagent	Vendor	Catalog #
n-Octane	Sigma	412236
37% Formaldehyde	EMD (VWR)	FX0410-5
Triton X-100	Sigma	T9284
10% Triton X-100	Roche	1332481

Source:

Methods in Cell Biology Volume 44, 1994

Drosophila melanogaster: Practical Uses in Cell and Molecular Biology

Edited by: Lawrence S.B. Goldstein / Eric A. Fyrberg

Chapter 25: Immunofluorescence Analysis of the Cytoskeleton during Oogenesis and Early Embryogenesis – William E. Theurkauf