

Materials List for:

Sonication-facilitated Immunofluorescence Staining of Late-stage Embryonic and Larval *Drosophila* Tissues *In Situ*

Ashley Fidler^{*1}, Lauren Boulay^{*1}, Matthew Wawersik¹

¹Department of Biology, College of William & Mary

*These authors contributed equally

Correspondence to: Matthew Wawersik at mjwawe@wm.edu

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Materials

Name	Company	Catalog Number	Comments
Table 1: Reagents and Buffers			
Phosphate buffer Triton X-100 (PBTx)			For 5 L: 500 ml PBS 10X, 4.45 L ddH ₂ O, 50 ml Triton 10%. Store at RT.
Phosphate buffer saline 10X (PBS 10X)			For 1 L in dH ₂ O: 80 g NaCl, 2 g KCl, 14.4g Na ₂ HPO ₄ , 2.4 g KH ₂ PO ₄ . Add components and fill to appropriate volume. Store at 4 °C.
Triton 10%			For 50 ml: 5 ml of Triton, 5 ml of PBS 10X, 45 ml of ddH ₂ O. Rock to mix. Store at RT.
PEMS			0.1 M Pipes (pH 6.9), 2.0 mM MgSO ₄ , 1.0 mM EGTA. Store at RT.
Pipes			For a 400 ml of a 0.25 M solution (pH 6.9): 30.24 g Pipes dH ₂ O NaOH. Dissolve Pipes in 300 ml dH ₂ O and then adjust to pH 6.9 with NaOH. Bring the total volume to 400 ml with dH ₂ O and autoclave. Store at RT.
Formaldehyde			37% formaldehyde by weight in methanol. Store at RT. Store formaldehyde, heptane, and methanol waste mixture in a tightly sealed container in fume hood before disposal as per institutional guidelines.
Heptane		CAS 142-82-5	n-Heptane. Store at RT. Store formaldehyde, heptane, and methanol waste mixture in a tightly sealed container in fume hood before disposal as per institutional guidelines.
Methanol		CAS 67-56-1	Store at RT. Store formaldehyde, heptane, and methanol waste mixture in a tightly sealed container in fume hood before disposal as per institutional guidelines.
Phosphate buffer Tween (PBTw)			To make 1 L: 100 ml PBS 10X, 890 ml ddH ₂ O, 10 ml Tween 10%. Filter sterilize after adding all components. Store at 4 °C.

Tween 10%			For 50 ml: 5 ml Tween, 5 ml of PBS 10X, 40 ml of ddH ₂ O. Rock to mix. Store at RT.
Bovine serum albumin/phosphate buffer Tween (BBTw)			To make 1 L: 100 ml PBS 10X, 890 ml ddH ₂ O, 10 ml Tween 10%, 1 g Bovine Serum Albumin (BSA). Add BSA then sterilize using a 0.2 μm vacuum filter unit. Store at 4 °C.
Normal goat serum (NGS)	ackson ImmunoResearch Laboratories	005-000-121	To make 10 ml: Normal goat serum 10 ml ddH ₂ O. Add ddH ₂ O to vial of NGS and sterilize using a 0.2 μm syringe filter. Store aliquots at -20 °C.
1,4-diazabicyclo[2.2.2]octane (DABCO)		CAS: 281-57-9	To make 100 ml: 25 ml ddH ₂ O, 1 ml Tris HCl (1M, pH 7.5), 2.5 g of DABCO solid, 3.5 ml 6N HCl, 250 μl 10N NaOH, 70 ml glycerol. In 250 ml beaker with stir bar, add ddH ₂ O, Tris HCl and DABCO. Stir and then add 6N HCl, 10 N NaOH, and glycerol. Then add 10N NaOH dropwise until solution reaches pH 7.5. Aliquot. Store aliquots at -20 °C.
DABCO + p-phenylenediamine (PPD) solution			1.765 ml NaHCO ₃ , 0.353 Na ₂ CO ₃ , 0.02 g PPD (CAS: 106-50-3). Dissolve PPD in NaHCO ₃ and NaCO ₃ solution. Add 60 μl of PPD solution to 500 μl of DABCO. Store aliquots at -20 °C.
Apple juice plates			To make ~200 plates: 45 g agar (CAS#9002-18-0), 45 g granulated sugar (store bought), 500 ml apple juice (store bought), 15 ml Tegosept 10%, 1.5 ml ddH ₂ O. Add agar to ddH ₂ O in 4 L flask then autoclave for 30 min. Mix apple juice and sugar on heated stir plate. Gradually add apple juice mixture to autoclaved agar. Mix on heated stir plate then aliquot 10 ml volumes into 35 mm petri dishes and let stand at RT to solidify. Store at 4 °C.
Tegosept 10%			To make 100 ml: 10 g Tegosept, 100 ml ethanol. Store aliquots at -20 °C.
Yeast paste			~50 g dry active yeast. Gradually add ddH ₂ O to beaker containing yeast while stirring until paste-like consistency reached. Store at 4 °C.

Table 2: Staining Materials

DAPI	Invitrogen	D3571	1:1000, stock at 5 mg/ml.
Rabbit anti-Vasa			1:250, a gift from Ruth Lehmann.
Mouse anti-Fasciclin III	Developmental Studies Hybridoma Bank (DSHB)	7G10	1:10
Mouse anti-1B1	Developmental Studies Hybridoma Bank (DSHB)	1B1	1:4
Guniea pig anti-Traffic Jam			1:2500, a gift from Dorthea Godt (Li et al, 2003).

Mouse anti-Prospero	Developmental Studies Hybridoma Bank (DSHB)	Prospero MR1A	1:10
Rat anti-Elav	Developmental Studies Hybridoma Bank (DSHB)	7EBA10	1:30
mouse anti-Repo	Developmental Studies Hybridoma Bank (DSHB)	8D12	1:10
Goat anti-rabbit Alexa546	Invitrogen	A11010	1:500
Goat anti-mouse Alexa488	Invitrogen	A11029	1:500
Goat anti-guinea pig Alexa633	Invitrogen	A21105	1:500
Goat anti-rat Alexa488	Invitrogen	A11006	1:500

Table 3: Materials and Equipment

Fly Cages	Hand-made; Genesee Scientific Corporation	Not applicable; Bottles: 32-130; Pre-made cage: 59-101	Made by cutting clear cast acrylic tubing (1 3/4 inch in diameter) into 4 inch tall segments with a compound miter saw at 400 rpm. Ultrafine stainless steel screening (was attached to one end of the tub with acrylic compound glue. An alternate method using an empty fly food bottle can be found in <i>Drosophila</i> Protocols ISBN 0-87969-584-4. Cages may also be purchased from the Genesee Scientific Corporation.
Sonicator: Branson 250 Digital Sonifier	Branson	Model: Branson Digital Sonifier 250	
Sonicator probe	Branson	Model #: 102C (CE)	EDP: 101-135-066; S/N: OBU06064658
Syringe filter	Nalgene	190-25-20	0.2 µm cellulose acetate membrane filter
Imaging system: Spinning disc confocal microscope with multichromatic light source, digital CCD camera, and imaging software	Microscope: Olympus, Light source: Lumen Dynamics, Camera: Q-Imaging, Imaging Software: Intelligent Imaging Inc.		Microscope: BX51 equipped with DSU spinning disc, Light source: X-Cite 120Q, Camera: RETIGA-SRV, Imaging Software: Slidebook 5.0
Vacuum filter unit	Nalgene	450-0020	0.2 µm cellulose nitrate membrane filter