

Materials List for:

# Dissection of the *Drosophila* Pupal Retina for Immunohistochemistry, Western Analysis, and RNA Isolation

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## Materials

Name	Company	Catalog Number	Comments
Adobe Photoshop	Adobe		Image processing software
Bamboo splints, 6"	Ted Pella Inc	116	
Beta mercaptoethanol	Sigma-Aldrich	M3148	
Beta-glycerol phosphate	Sigma-Aldrich	50020	
Black dissecting dish			Glass petri dish filled to rim with SYLG170 or SYLG184 (colored black with finely ground charcoal powder). Leave at room temperature for 24-48 h to polymerize.
Blade holder	Fine Science Tools	10053	
Bovine serum albumin	Sigma-Aldrich	A7906	
cOmplete, EDTA-free protease inhibitor cocktail tablets	Roche	4693132001	
Confocal microscope (Zeiss LSM 501)	Carl Zeiss		or similar microscope
Diethyl Pyrocarbonate (DEPC)	Sigma-Aldrich	40718	
Double-sided tape	3M	665	
<i>Drosophila</i> food media, nutrient-rich			7.5% sucrose, 15% glucose, 2.5% agar, 20% brewers yeast, 5% peptone, 0.125% MgSO <sub>4</sub> ·7H <sub>2</sub> O, 0.125% CaCl <sub>2</sub> ·2H <sub>2</sub> O
<i>Drosophila</i> food media, standard			Bloomington <i>Drosophila</i> Stock center cornmeal recipe. ( <a href="https://bdsc.indiana.edu/information/recipes/bloomfood.html">https://bdsc.indiana.edu/information/recipes/bloomfood.html</a> )
Ethylenediaminetetraacetic acid	Sigma-Aldrich	E6758	
Fixative solution			4% formaldehyde in PBS, pH 7.4.
Fluorescence microscope (TCS SP5 DM microscope)	Leica Microsystems		or similar microscope
Forceps	Fine Science Tools	91150-20	Forceps should be sharpened frequently.
Formaldehyde	Thermo Scientific	28908	
Glass 9-well dishes	Corning	7220-85	Also known as 9-well dishes
Glass coverslips (22 x 22 mm)	Fisher Scientific	12-542-B	
Glass microscope slides (25 x 75 x 1 mm)	Fisher Scientific	12-550-413	
Glass petri dish	Corning	3160-100BO	
Glycerol	Sigma-Aldrich	G5516	

Image Studio software version 5.2.5	LI-COR Biosciences		Image processing software for quantitation of Western blots.
Laemmli sample buffer	Bio-Rad	161-0737	2X concentrated protein sample buffer, supplement with beta mercaptoethanol as per manufacturer's instructions.
Lane marker reducing sample buffer	ThermoFisher Scientific	39000	5X concentrated protein sample buffer.
Microcentrifuge tubes	Axygen	MCT-175-C	
Microdissection scissors	Fine Science Tools	15000-03	
Microwell trays (72 x 10 $\mu$ L wells)	Nunc	438733	
Mounting media			0.5% N-propylgallate and 80% glycerol in PBS
N-propylgallate	Sigma-Aldrich	P3130	
Nuclease-free PBS (PBS in 0.1% DEPC, pH 7.4)			Add appropriate volume of DEPC to PBS, mix well and incubate overnight at room temperature with constant stirring. Autoclave for at least 20 minutes. Store at 4°C
PBS (phosphate buffered saline pH 7.4)	Sigma-Aldrich	P5368	Prepare according to manufacturer's instructions
PBS+pi (PBS plus protease and phosphatase inhibitors)			10mM NaF, 1mM beta-glycerol phosphate and 1mM Na <sub>3</sub> VO <sub>4</sub> in PBS, pH 7.4.
PBT			0.15% TritonX and 0.5% bovine serum albumin in PBS, pH 7.4
Pin holder	Fine Science Tools	26016-12	
Primary antibody: goat anti-GAPDH	Imgenex	IMG-3073	For Western blotting. Used at 1:3000
Primary antibody: rabbit anti-cleaved Dcp-1	Cell signaling	9578S	For immunofluorescence. Used at 1:100
Primary antibody: rat anti-DEcad	Developmental Studies Hybridoma Bank	DCAD2	For immunofluorescence. Used at 1:20
Primary antibody: rat anti-DEcad	DOI: 10.1006/dbio.1994.1287	DCAD1	Gift from Tadashi Uemura. Used at 1:100.
RNA extration kit: Relia Prep RNA tissue Miniprep kit	Promega	Z6110	
Rnase decontamination reagent (RNase Away)	Molecular BioProducts	7002	
Scapel blades	Fine Science Tools	10050	Break off small piece of scapel blade and secure in blade holder.
Secondary antibody: 488-conjugated donkey anti-rat IgG (H+L)	Jackson ImmunoResearch	712-545-153	For immunofluorescence. Used at 1:200
Secondary antibody: cy3-conjugated goat anti-rabbit IgG (H+L)	Jackson ImmunoResearch	111-165-144	For immunofluorescence. Used at 1:100
Secondary antibody: HRP-conjugated goat anti-rat IgG (H+L)	Cell Signaling Technology	7077	For Western blotting. Used at 1:3000
Secondary antibody: HRP-conjugated rabbit anti-goat IgG (H+L)	Jackson ImmunoResearch	305-035-003	For Western blotting. Used at 1:3000
Sodium Chloride	Sigma-Aldrich	S3014	
Sodium Fluoride	Sigma-Aldrich	215309	
Sodium vanadate	Sigma-Aldrich	50860	

Spectrophotometer (NanoDrop)	ThermoFisher Scientific	2000c	
Stereo dissecting microscope (M60 or M80)	Leica Microsystems		or similar microscope
Sylgard (black)	Dow Corning	SYLG170	
Sylgard (transparent)	Dow Corning	SYLG184	Color black with finely ground charcoal powder
Tissue: Kimwipes	KIMTECH	34120	
TritonX	Sigma-Aldrich	T8787	
Trizma hydrochloride pH7.5	Sigma-Aldrich	T5941	
Tungsten needle, fine	Fine Science Tools	10130-10	Insert into pin holder
Tungsten needle, sturdy	Fine Science Tools	10130-20	Insert into pin holder
WTLB (western tissue lysis buffer)			150mM NaCl, 1.5% Triton X-100, 1mM EDTA, 20% glycerol, 10mM NaF, 1mM beta-glycerol phosphate and 1mM Na <sub>3</sub> VO <sub>4</sub> in 50mM Tris-HCl (pH 7.5). Supplement with one cOmplete protease cocktail table per 10 mL solution.
Yeast paste	(local supermarket)		Approximately 2 tablespoons Fleischmann's ActiveDry Yeast (or similar) dissolved in ~20 mL distilled H <sub>2</sub> O