

Materials List for:

Preparation of Mouse Retinal Cryo-sections for Immunohistochemistry

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Materials

Name	Company	Catalog Number	Comments
6qt. Stainless Steel Beaker (185 x 218mm)	Gilson	#MA-48	For liquid nitrogen (Figure 1E)
Aluminum foil			
Cauterizer	Bovie	#AA01	To mark eye orientation (Figure 1A)
Curved forceps, Dumont #5/45 tweezers, 45-degrees bent	Electron Microscopy Sciences	#72703-D	For mouse eye enucleation and maintenance (Figure 1A)
Curved scissors, Vannas Spring Scissors - 2.5mm Cutting Edge	Fine Science Tools	#15002-08	To circumferentially cut the cornea (Figure 1B)
Dental wax-coated 35mm dissection dish			For eye cup dissection (Figure 1C)
Dissecting microscope	Leica, Houston, USA	MZ12.5 High-performance stereomicroscope	
Micro Knives - Plastic Handle	Fine Science Tools	#10315-12	To make a small incision at the burn mark (Figure 1A)
Pink dental wax	Electron Microscopy Sciences	#72660	For coating dissection dish
Slide Rack and Coverplate	Ted Pella	#36107	For retinal IHC (Figure 1G)
Stainless Steel Beaker (89 x 114mm)	Gilson	#MA-40	For isopentane bath (Figure 1E)
Styrofoam box			For insulated cooler
Thin forceps Dumont #5	Fine Science Tools	#11254-20	To remove the cornea and extract the lens (Figure 1B)
Tissue-Tek cryomold (10mm x 10mm x 5mm)	Electron Miscroscopy Sciences	#62534-10	Mold for OCT embedding
Buffers and Reagents	Company	Catalog Number	Comments
Blocking solution			2% Normal Horse Serum, 1.5% Cold Fish skin Gelatin (at 40-50% in H2O, cat #G7765), 5% bovine serum albumin (cat #AK1391-0100) in permeabilization buffer.
Gelvatol (anti-fading mounting media)			Under the fume hood, dissolve 0.337g DABCO (Sigma cat #D2522-25G) in 10mL Fluoromount G (Fisher cat #OB100-01). Adjust to pH 8-8.5 with 12N HCl (~ 5 drops). Store in amber drop bottle at 4°C (8).
Hoechst 33342 1000x Stock	Thermo Scientific	#62249	1 mg/ml in PBS (1000x). Aliquot and store in dark, at 4°C for up to 1 year or at -20°C for long term storage. Prepare fresh at 1 ug/mL (1x)

Isopentane, 99%	GFS Chemicals	#2961	
Liquid Nitrogen			
Paraformaldehyde (PFA) in PBS	Electron Microscopy Sciences	#15710	Prepared fresh 4% PFA from 16% PFA stock. Store the remaining 16% PFA at 4°C in the dark.
Permeabilization solution			PBS + 0.25% Triton-X (AMRESCO cat #0694-1L) + 0.05% NaN ₃ . Prepare fresh.
Phosphate-buffered saline (PBS)	Bioworld	#41620015-20	0.02 g/L KCl, 0.02 g/L KH ₂ PO ₄ , 0.8 g/L NaCl, 0.216 g/L Na ₂ HPO ₄ , pH 7.4. Prepared from 10x stock
Sucrose solutions	Fisher Scientific	#S-5-500	Dissolve the appropriate amount of sucrose in 37°C PBS (takes ~15 min to dissolve). May be stored for a few days at 4°C.
Tissue-Tek OCT-compound	Sakura	#4583	
Antibodies	Company	Catalog Number	Comments
anti-calbindin	Sigma-Aldrich	C8666	To label horinzotal cells
anti-GAD65	Chemicon	AB5082	To label GABAergic amacrine cells
anti-GS	BD Transduction	610517	To label Müller cells
anti-rhodopsin	Millipore	MAB5316	To label rods