

A Protocol to Evaluate and Quantify Retinal Pigmented Epithelium Pathologies in Mouse Models of Age-Related Macular Degeneration

Michael Landowski^{1,2}, Samuel Grindel¹, Ying Hao³, Sakae Ikeda^{1,2}, Catherine Bowes Rickman^{3,4}, Akihiro Ikeda^{1,2}

¹Department of Medical Genetics, University of Wisconsin-Madison ²McPherson Eye Research Institute, University of Wisconsin-Madison ³Department of Ophthalmology, Duke University ⁴Department of Cell Biology, Duke University

Corresponding Author

Akihiro Ikeda
aikeda@wisc.edu

Citation

Landowski, M., Grindel, S., Hao, Y., Ikeda, S., Bowes Rickman, C., Ikeda, A. A Protocol to Evaluate and Quantify Retinal Pigmented Epithelium Pathologies in Mouse Models of Age-Related Macular Degeneration. *J. Vis. Exp.* (193), e64927, doi:10.3791/64927 (2023).

Date Published

March 10, 2023

DOI

10.3791/64927

URL

jove.com/video/64927

Materials

Name	Company	Catalog Number	Comments
0.1 M Cacodylate Buffer pH7.2	PolyScientific R&D Company	S1619	
100 Capacity Slide Box			Two are needed for this protocol (one for H&E-stained slides and one for RPE flat mounts.)
100% Ethanol	MDS Warehouse	2292-CASE	Can be used to make diluted ethanol solutions in this protocol.
1-Way Stopcock, 2 Female Luer Locks	Qosina	11069	
1x Phosphate Buffer Solution (PBS)			Premade 1x PBS can be used in this protocol.
2.0 mL microtubes	Genesee Scientific	24-283-LR	
24 Cavity Embedding Capsule Substitute Mold	Electron Microscopy Sciences	70165	
24 inch PVC Tubing with Luer Ends	Fisher Scientific	NC1376778	
400 Mesh Gilder Thin Bar Square Mesh Grids	Electron Microscopy Sciences	T400-Cu	
95% Ethanol	MDS Warehouse	2293-CASE	
Absorbent Underpads with Waterproof Moisture Barrier (23 inches by 24 inches)	VWR	56616-031	
Adjustable 237 ml Spray Bottle	VWR	23609-182	
Alexa Fluor488 Conjugated Donkey anti-Rabbit IgG	Thermo Fisher Scientific	A-21206	
Aluminum Foil			
BD Precision glide 19 Gauge Syringe Needle	Sigma-Aldrich	Z192546	
Bracken Forceps; Curved; Fine Cross Serrations; 4" Length, 1 mm Tip Width	Roboz Surgical Instrument	RS-5211	Known as curved forceps in this protocol.

Camel Hair Brush	Electron Microscopy Sciences	65575-02	
Carbon Dioxide Euthanasia Chamber			
Carbon Dioxide Flow Meter			
Carbon Dioxide Tank			
Castaloy Prong Extension Clamps	Fisher Scientific	05-769-7Q	
Cast-Iron L-shaped Base Support Stand	Fisher Scientific	11-474-207	
Cell Prolifer Program			Available to download: https://cellprofiler.org/releases
Clear Nail Polish	Electron Microscopy Sciences	72180	
Colorfrost Microscope Slides Lavender	VWR	10118-956	
Computer			
DAPI	Sigma-Aldrich	D9542-5MG	
Distilled H ₂ O			Water from Milli-Q Purification System was used in this protocol.
Dumont Thin Tip Tweezers; Pattern #55	Roboz Surgical Instrument	RS-4984	Known as fine-tipped forceps in this protocol, and 3 are needed for this protocol (two for dissections and one for electron microscope processing).
Electron Microscopy Grid Holder	Electron Microscopy Sciences	71147-01	
EPON 815 Resin	Electron Microscopy Sciences	14910	
Epredia Mark-It Tissue Marking Yellow Dye	Fisher Scientific	22050460	Please follow manufacturer's protocol when using this tissue marking dye.
Epredia Mounting Media	Fisher Scientific	22-110-610	Use for mounting H&E slides.
Fiber-Lite Mi-150 Illuminator Series, 150 w Halogen Light Source	Dolan-Jenner Industries	Mi-150	Light source for dissecting microscope.
Fiji ImageJ Program			Available to download: https://imagej.net/downloads
Flexaframe Castaloy Hook Connector	Thermo Scientific	14-666-18Q	
Fume hood			
Glutaraldehyde 2.5% in Phosphate Buffer, pH 7.4, 32%	Electron Microscopy Sciences	16537-05	
JEM-1400 Transmission Electron Microscope (JEOL) with an ORIUS (1000) CCD Camera			
Laboratory Benchtop Shaker			Two are needed for these experiments. One should be at room temperature while the other should be in a 4 degree Celsius cold room.
Laser Cryo Tag Labels	Electron Microscopy Sciences	77564-05	
Lead Citrate	Electron Microscopy Sciences	17800	
Leica EM UC7 Ultramicrotome			
Leica Reichert Ultracut S Microtome			
LifterSlips	Thermo Fisher Scientific	22X22I24788001LS	Use these coverslips for the RPE flat mounts as they have raised edges and accommodate the thickness of the RPE.
Mayer's Hematoxylin	VWR	100504-406	
McPherson-Vannas Micro Dissecting Spring Scissors	Roboz Surgical Instrument	RS-5600	Known as micro-dissecting scissors in protocol.
Methanol	Fisher Scientific	A412-4	

Mice			Any AMD mouse model and its respective controls can work for this protocol.
Micro Dissecting Scissors; Standard Version; Curved; Sharp Points; 24 mm Blade Length; 4.5" Overall Length	Roboz Surgical Instrument	RS-5913	Known as curved scissors in this protocol.
Microsoft Excel			
Microtube racks			
Nikon A1RS Confocal Microscope			
Normal Donkey Serum	SouthernBiotech	0030-01	
Number 11 Sterile Disposable Scalpel Blades	VWR	21909-380	
Osmium Tetroxide	Electron Microscopy Sciences	19150	
Paraformaldehyde, 32%	Electron Microscopy Sciences	15714-S	
Pencil			
Petri Dish	VWR	21909-380	
Pipette Tips			
Pipettes			
Polyclonal Anti-ZO-1 Antibody	Thermo Fisher Scientific	402200	
Propylene Oxide	Electron Microscopy Sciences	20412	
Razor Blade	VWR	10040-386	
Shallow Tray for Mouse Perfusions			
Shandon Eosin Y Alcoholic	VWR	89370-828	
Sharpie Ultra Fine Tip Black Permanent Marker	Staples	642736	
Slide Rack for Staining	Grainger	49WF31	
Squared Cover Glass Slips	Fisher Scientific	12-541B	
Staining Dish with Cover	Grainger	49WF30	Need 15 for H&E staining procedure.
Target All-Plastic Disposable Luer-Slip 50 mL Syringe	Thermo Scientific	S7510-50	Use only the syringe barrel.
Timer	Fisher	1464917	
Uranyl Acetate	Electron Microscopy Sciences	22400	
Vacuum Oven			
Vectashield Mounting Medium	Vector Laboratories	H-1000	Use for mounting RPE flat mounts.
Xylene	Fisher Scientific	22050283	
Zeiss Axio Imager 2 Light Microscope			This microscope has the capacity to generate stitched 20x images. If a light microscope does not have this capacity, then take images of the entire retina that are slightly overlapping each other. Use Adobe Photoshop to stitch these images together. Please refer to the manuals of the Adobe Photoshop program for image stitching.
Zeiss Stemi 2000 Dissecting Microscope	Electron Microscopy Sciences	65575-02	