

Materials List for:

# Analysis of Nephron Composition and Function in the Adult Zebrafish Kidney

Kristen K. McCampbell<sup>1</sup>, Kristin N. Springer<sup>1</sup>, Rebecca A. Wingert<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, University of Notre Dame

Correspondence to: Rebecca A. Wingert at [rwingert@nd.edu](mailto:rwingert@nd.edu)

URL: <https://www.jove.com/video/51644>

DOI: [doi:10.3791/51644](https://doi.org/10.3791/51644)

## Materials

Name	Company	Catalog Number	Comments
1X PBS			Made by diluting 10 X PBS in distilled water.
1X PBST			0.1% Tween-20 detergent in 1 X PBS.
1X PBS with 0.05 % Tween			0.05% Tween-20 detergent in 1 X PBS.
Tween 20	American Bioanalytical	AB02038	
4% PFA/1X PBS			Dissolve 4% PFA (w/v) (Electron Microscopy Sciences, Cat # 19210) in 1 X PBS, bring to boil on a hot plate in a fume hood. Cool and freeze the aliquots for storage in the freezer at -20°C. Thaw just before use and do not refreeze stocks.
Fine Forceps	Roboz	RS-1050	Dumont Tweezers Pattern #55
Glass slide	Thermo-Fisher	4445	White Frost
Glass coverslip	Thermo-Fisher	12-540A	18 x 18 mm
Modeling clay	Hasbro	Playdoh	Other modeling clays can be substituted and work similarly
Slide holder	Thermo-Fisher	12-587	Optional: cardboard tray to store slides flat
Bleaching solution			Bleach mix formula (for a 20 ml solution): 1.6 ml of 10% Potassium hydroxide solution 0.6 ml of 30% Hydrogen peroxide solution 100 µl of 20% Tween Fill to 20 ml with distilled water
Potassium hydroxide	Sigma	221473	
Hydrogen peroxide	Sigma	H1009	
Blocking solution			Blocking Solution (for a 10 ml solution): 8 ml of 1X PBS with 0.05% Tween 2 ml of fetal calf serum 150 µl of DMSO
Alkaline phosphatase activity detection	Invitrogen	E6601	We utilize the ELF 97 Endogenous Phosphatase Detection Kit. To prepare the working substrate solution, dilute the substrate 20-fold in the detection buffer, according to the manufacturer's instructions.

Alkaline phosphatase wash solution			Recipe for Wash Buffer Solution (for a 100 ml solution): 5 ml of 0.5 M EDTA 120 mg of Levamisole 95 ml of 1X PBS
Dextran, fluorescein, 40,000 MW	Invitrogen	D1844	Dilute with distilled water to make a 50 mg/ml stock solution, and store aliquots in the freezer at -20°C.
Dextran, cascade blue, 10,000 MW	Invitrogen	D1976	Dilute with distilled water to make a 50 mg/ml stock solution, and store aliquots in the freezer at -20°C.
Dextran, lucifer yellow, 10,000 MW	Invitrogen	D1825	Dilute with distilled water to make a 50 mg/ml stock solution, and store aliquots in the freezer at -20°C.
Dextran, tetramethylrhodamine (fluoro-ruby), 10,000 MW	Invitrogen	D1817	Dilute with distilled water to make a 50 mg/ml stock solution, and store aliquots in the freezer at -20°C.
Dolichos biflorus agglutinin (DBA)-rhodamine	Vector laboratories	RL-10-32	DBA Staining Solution (for a 100 µl solution): 1 µl of DBA 99 µl of 1X PBS
Propidium iodide	Invitrogen	E6601	
DAPI	Invitrogen	P1304MP	
Vectashield hard set mounting medium	Vector laboratories	H-1400	
Micro cover glass	VWR	48393081	
Dimethyl sulfoxide, DMSO	American Bioanalytical	67-68-5	
Sucrose	Sigma	S0289	
EDTA, 0.5 M solution, pH 8.0	American Bioanalytical	AB00502	
Levamisole	Sigma	L-9756	
Tissue freezing medium	Triangle Biomedical Sciences, Inc.	TFM-C	
Tissue-Tek Cryomold Biopsy, 10 x 10x 5 mm	Sakura Finetek	4565	
TruBond 380 adhesive microscope slides	Tru Scientific	0380W	
Liquid blocker – super PAP pen	Electron Microscopy Sciences	71312	
Immuno stain moisture chamber	Evergreen Scientific	240-9020-Z10	
Cryostat	Therm	Microm™ HM 525 Cryostat	
Stereomicroscope	Nikon	SMZ645; SMZ1000; 83455 P-Blue GFP/DAPI; 83457 P-Endow GFP/FITC; 83457 P-TRITC	Filter set used was as follows: Hoechst/DAPI filter was used for DAPI, propidium iodide, dextran-cascade blue and alkaline phosphatase detection. GFP/FITC filter was used for dextran-FITC, dextran lucifer yellow, and anti-GFP detection. The TRITC filter was used for dextran-fluoro-ruby, PI, and DBA detection.
Compound microscope	Nikon	96310 C-FL UV-2E/C DAPI; 96311 C-FL B-2E/C FITC; 96313 C-FL Y-2E/C Texas Red	Filter set used was as follows: Hoechst/DAPI filter was used for DAPI, propidium iodide, dextran-cascade blue and alkaline phosphatase detection. GFP/FITC filter was used for dextran-FITC, dextran lucifer yellow, and anti-GFP detection. The Texas Red filter was used for dextran-fluoro-ruby, PI, and DBA detection.