

Materials List for:

Sequential Immunofluorescence and Immunohistochemistry on Cryosectioned Zebrafish Embryos

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Materials

Name	Company	Catalog Number	Comments
15/25	N/A	N/A	15% fish gelatin/25% sucrose w/v, made in dH ₂ O
Alexa 555 secondary antibody	Invitrogen	A21429	used at 1:2,000 dilution in block buffer for IF
Antibody Diluent	Dako	S3022	
Background Buster	Innovex	NB306	
block buffer (IF)	N/A	N/A	5% normal goat serum, 0.1% Triton X-100 in 1x PBS
cellSens imaging software	Olympus	cellSens	imaging software used with compound light microscope
chimeric zebrafish embryos	N/A	N/A	AB wild-type chimera zebrafish embryos analyzed at 48 hpf
Compound light microscope	Olympus	BX51	used with digital camera and cellSens imaging software for image capture after IHC
Confocal fluorescence microscope	Leica	DM 2500	used with digital camera and Leica Application Suite Advanced Fluorescence imaging software for image capture after IF
Disposable plastic molds, 15 mm x 15 mm x 5 mm	Ted Pella	27147-2	
Digital camera, light microscope	Olympus	DP27	
Digital camera, fluorescence microscope	Leica	TCS SPE	
Ethanol	Koptec	V1001	
Fish gelatin	Sigma	G7041	
Glass slides	Fisher	12-550-15	
Hydrogen peroxide, 30%	Fisher	H325-100	
ImmPRESS HRP Polymer detection kit	Vector	MP-7401	anti-rabbit secondary antibody, HRP polymer
Leica Application Suite Advanced Fluorescence imaging software	Leica	LAS AF 2.3.6	imaging software used with confocal fluorescence microscope
Methanol	Fisher	A452-4	
Modified Meyer's Hematoxylin	Thermo	72804	
Normal Goat Serum	MP Biomedical	191356	
OCT medium	Tissue-Tek/Fisher	4583/4585	
anti-Oregon Green antibody	Molecular Probes/Life Technologies	A889	used at 1:7500 dilution for IHC
Oregon Green Dextran	Invitrogen	P7171	used at 2.5% in 0.2 M KCl

Paraformaldehyde, 4%	Acros Organics	416780030	made in lab in 1x PBS
Permunt	Fisher	SP15	
1x phosphate-buffered saline	made in lab	N/A	50 mL 10x PBS, up to 500 mL volume with dH ₂ O
10x phosphate-buffered saline	made in lab	N/A	use Cold Spring Harbor protocol
1x PBSt	made in lab	N/A	50 mL 10x PBS, 1 mL 10% Tween 20, up to 500 mL volume with dH ₂ O
anti-Ser10 phosphorylated Histone H3 antibody	Santa Cruz	sc-8656-R	used at 1:500 dilution for IF
Scott's Tap Water	Electron Microscopy Sciences	26070-06	have used other brands successfully in addition to EMS
Sucrose	Amresco	335	
Tween-20	Fisher	BP337	
TO-PRO3	Invitrogen	T3605	used at 1:1,000 in 1x PBS for IF
1x Tris-buffered saline	made in lab	N/A	50 mL 10x TBS, up to 500 mL volume with dH ₂ O
10x Tris-buffered saline	made in lab	N/A	85 g NaCl, 61 g Tris base, up to 1 L volume with dH ₂ O
1x TBSt	made in lab	N/A	50 mL 10x TBS, 1 mL 10% Tween 20, up to 500 mL volume with dH ₂ O
Triton X-100	Acros Organics	327371000	
Vectashield	Vector	H-1000	non-hardening mounting media
Vector NovaRed substrate	Vector	SK-4800	HRP substrate
Xylene	Fisher	X3P-1GAL	