

Materials List for:

Visualizing Multiciliated Cells in the Zebrafish Through a Combined Protocol of Whole Mount Fluorescent *in Situ* Hybridization and Immunofluorescence

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Materials

Name	Company	Catalog Number	Comments
non-specific protease mixture solution	Roche	11459643001, pronase from <i>Streptomyces griseus</i>	Dilute in E3 without methylene blue to make 50mg/mL stock solution; store at -20°C
E3 solution			Dilute 50X E3 (250 mM NaCl, 8.5 mM KCl, 16.5 mM CaCl ₂ , 16.5 mM MgSO ₄ in distilled water) in distilled water; add 1 x 10 ⁻⁵ M methylene blue (sigma M9140) to inhibit contamination
tricaine (ethyl 3-aminobenzoate methanesulfonate)	Fluka	A5040-250G	To make a 0.2% stock solution, dissolve 1 g of tricaine powder in 10 mL Tris, pH 9.5 and distilled water up to 500 mL
embryo dishes	VWR	351029	
5 mL glass vials	Wheaton	225012	
disposable plastic Pasteur pipettes	VWR	414004-004	
4% paraformaldehyde (PFA) solution	Electron Microscopy Services	19210	Dissolve 4% PFA in 1X PBS and bring to a boil under a fume hood. Cool and aliquot, then store at -20°C. Do not refreeze once thawed for use.
10X PBS	American Bioanalytical	AB11072	Dilute in distilled water to make a 1X stock
Tween-20 stock	American Bioanalytical	AB02038	Make a 0.1% Tween-20 stock by diluting in distilled water.
1X phosphate buffered saline with 0.1% Tween 20 (PBST)	Sigma	P9416	0.1% Tween-20 in 1X PBS
methanol (MeOH)	Sigma	34860-4L	
proteinase K	Roche	3115879001	Dissolve in distilled water to make a 10 mg/mL stock; aliquot and store at -20°C
flat bottom microcentrifuge tubes	VWR	87003-300; 87003-298	
formamide	American Bioanalytical	AB00600	store at -20°C
hybridization solution (HYB+)			50% formamide, 5X SSC, 0.1% Tween-20, 5 mg/mL yeast torula RNA, 50 µg/µL heparin; store at -20°C
hybridization oven	Fisher Scientific	13-247-10Q	
20X saline-sodium citrate (SSC) buffer solution	American Bioanalytical	AB13156	dilute in distilled water to make 2X and 0.2X stocks

blocking reagent solution	Roche	11096176001	dilute in maleic acid buffer to make a 10% stock solution; store at 4°C
maleic acid buffer solution	Sigma	M0375 and S7653	150mM maleic acid, 100mM NaCl (pH 7.5)
anti-Digoxigenin-POD, Fab fragments	Roche	11207739910	store at 4°C
Cy3 fluorescent staining solution	PerkinElmer, Inc.	NEL744001KT, TSA Plus Cyanin3 system	store at 4°C; prepare staining solution fresh by making a 1:50 dilution of TSA reagent (dissolved in 60 uL of DMSO) in the kit buffer
Hydrogen peroxide (H2O2)	Sigma	H1009-500mL	store at 4°C
molecular grade distilled water (ddH2O)	Mediatech	25-055-CM	
acetone	American Bioanalytical	AB00636-01000	store an aliquot at -20°C
DMSO	American Bioanalytical	AB00435-01000	
fetal bovine serum (FBS)	Gibco	10438-034	aliquot and store at -20°C
monoclonal anti-acetylated tubulin clone 6-11B-1	Sigma-Aldrich	T6793	aliquot and store at -20°C
anti-γ-tubulin anitbody produced in rabbit	Sigma-Aldrich	T5192	aliquot and store at -20°C
odf3b cDNA clone MGC:63985	OpenBiosystems	IMAGE:6792478	store bacterial glycerol stock at -80°C
NaCL	American Bioanalytical	AB01915-05000	
Alexa flour 647 goat anti-rabbit IgG	Life Technologies	A21245	store at 4°C; protect from light
Alexa fluor 488 goat anti-mouse IgG	Life Technologies	A11029	store at 4°C; protect from light
DAPI	Life Technologies	D1306	aliquot and store at -20°C
glass slide	Thermo-Fisher	4445	
glass coverslip	Thermo-Fisher	12-540A	18 x 18 mm
fine forceps	Roboz	RS-1050	Dumont Tweezers Pattern #55
mounting media	Polysciences, Inc.	18606, Aqua-Poly/Mount	store at 4°C
confocal microscope and associated software			We use a Nikon C2plus Confocal Microscope with NIS-elements AR software
rocker	Bio-Rad	1660710EDU	