Materials List for:

Flat Mount Preparation for Observation and Analysis of Zebrafish Embryo Specimens Stained by Whole Mount *In situ* Hybridization

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Materials

Name	Company	Catalog Number	Comments
50 X E3			250 mM NaCl, 8.5 mM KCl, 16.5 mM $CaCl_2$, 16.5 mM $MgSO_4$ in distilled water. Supplement with methylene blue (1 x 10^{-5} M) (Sigma M9140) to inhibit contamination.
1 X E3			Dilute 50 X E3 in distilled water.
Mesh tea strainer	English Tea Store	SKU #ASTR_KEN, MPN#1705	
Embryo incubation dish	Falcon	35-1005	
Transfer pipette	Samco	202, 204	
Flat bottom microcentrifuge tube	VWR	87003-300; 87003-298	
Glass vial	Wheaton	225012	
1x PBST			0.1% Tween-20 detergent in 1x PBS, made by diluting 10x PBS in distilled water.
Tween-20 stock	American Bioanalytical	AB02038	
10x PBS	American Bioanalytical	AB11072	
Paraformaldehyde	Electron Microscopy Services	19210	
4% PFA/1X PBS			Dissolve 4% PFA (w/v) in 1x PBS, bring to boil on a hot plate in a fume hood. Cool and freeze aliquots for storage at -20 °C. Thaw just before use and do not refreeze stocks.
Filter sterilization unit	Corning	430516	1 L filter system, 0.45 µm CA
MeOH	Sigma	34860-4L	
Proteinase K	Roche	03-115-879-001	Dissolve in distilled water to make proteinase K stock (10 mg/ml) and store aliquots at -20 °C.
HYB+			50% formamide, 5x SSC, 0.1% Tween-20, 5 mg/ml yeast torula RNA, 50 µg/µl heparin
DIG/FLU labeled riboprobe *in vitro* transcription reaction reagents	Roche	11175025910; 11685619910	Refer to manufacturer instructions.
SP6 RNA polymerase	Roche	11487671001	
T7 RNA polymerase	Roche	10881775001	
T3 RNA polymerase	Roche	11031171001	
RNase 1 inhibitor	Roche	3335399001	
DNase 1 inhibitor, 10x DNase 1 buffer	Roche	4716728001	

Glycogen	Roche	10901393001	
Ethanol (EtOH)	Sigma	E7023	Aliquot 50 ml aliquots of 100% EtOH and 70% EtOH (diluted with molecular grade water) and store at -20 °C.
Molecular grade distilled water	Mediatech	25-055-CM	
Waterbath	Thermo	51221073	Model 2831
Nanodrop	Thermo	ND-2000c	
Formamide	American Bioanalytical	AB00600	Store at -20 °C.
20x SSC	American Bioanalytical	AB13156	
MAB			2 L formula: Into 1.5 L of distilled water, mix 23.2 g maleic acid, 17.5 g NaCl, 55.0 g Trisma base, then add 8 ml of 1M Tris-HCl pH 9.5, and then fill to 2 L. Autoclave.
MABT			MAB + 0.1% (v/v) Tween-20
Block solution			We make in 50 ml fresh aliquots: 10 ml of BSA (from 10% lab stock), 5 ml of FCS (from stock), and 35 ml of MABT. Save unused block at 4 °C to use for antibody solution. Note: Thaw the BSA and FCS stocks at 37 °C–if you thaw at a higher temperature they become a thick gel (do not use).
FCS (fetal calf serum) stock	Invitrogen	16140089	Aliquot in 5 or 10 ml portions and store at -20 °C.
BSA (bovine serum albumin) stock	American Bioanalytical	AB00448	Make a 10% stock diluted in MAB by dissolving the BSA flakes at room temperature with rapid stirring, then store 10 ml aliquots at -20 °C. Store undissolved BSA flakes at 4 °C.
Anti-digoxigenin antibody	Roche	11-093-274-910	Store at 4 °C.
Anti-fluorescein antibody	Roche	11-426-338-910	Store at 4 °C.
12-well staining dish	BD-Falcon	35-3225	
Pre-staining buffer			100 mM Tris pH 9.5, 50 mM $MgCl_2$, 100 mM NaCl, 0.1% (v/v) Tween-20. Always make fresh: it will precipitate in the course of a few days.
Staining solution-purple			For every 10 ml needed: add 45 µl of NBT and 35 µl of BCIP to fresh pre-staining buffer (not used on embryo samples).
Staining solution-red			For every 10 ml needed: add 31.5 µl of INT and 35 µl of BCIP to fresh pre-staining buffer (not used on embryo samples).
NBT stock	Sigma	N6876	Stored at -20 °C; powder diluted 50 mg/ml in 70% DMF, 30% water.
INT stock	Sigma	I8377	Stored at -20 °C; powder diluted 55 mg/ml in 70% DMF, 35% water.
BCIP stock	Sigma	B8503	Stored at -20 °C; powder diluted 50 mg/ml in 100% DMF.
DMF (dimethylformaldehyde)	American Bioanalytical	AB00450	
Glycine	Sigma	G8898	0.1 M glycine, pH 2.2

Small plastic Petri dish	Corning	430589, 430588	
Glycerol	Sigma	G7893	
Fine forceps	Roboz	RS-1050	Dumont Tweezers Pattern #55
Lash tool			Constructed by affixing a suitable lash to a pipette tip (sizes ranging from P10-P1000 can be used) using superglue. We use a naturally shed human eyelash, a naturally shed animal whisker or wiry fur coat hair, or a natural or synthetic lash purchased from the beauty department at a pharmaceutical or retail store (extra long lashes are most amenable to stable mounting on the pipette tip). The pipette tip is affixed onto a straight rod (8-10 cm in length) such as a needle holder (*e.g.*, Fisher 08-965-A) for easy handling. See **Figure 3** for images of these homemade tools.
Glass slide	Thermo-Fisher	4445	White Frost
Glass coverslip	Thermo-Fisher	12-540A	18 x 18 mm
Modeling clay	Hasbro	Playdoh	Other craft modeling clay products can be substituted.
Slide holder	Thermo-Fisher	12-587-10	Cardboard tray to store slides flat.
Stereomicroscope	Nikon	SMZ645, SMZ1000	
Compound microscope	Nikon	80i, 90i	