

A Label-free Xenograft Model for Investigating the Behavior of Human Stem Cell Spheroids in Chick Embryos

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Materials

Name	Company	Catalog Number	Comments
Animals			
Gallus gallus eggs	Granja Tolomei	SPF-free	White leghorn chicken
Reagents			
Alcian Blue 8GX	Sigma-aldrich	A5268	
AluFw primers	Sigma-aldrich	OLIGO	5'-CGA GGC GGG TGG ATC ATG AGG T-3'
AluRev primers	Sigma-aldrich	OLIGO	5'-TTT TTT GAG ACG GAG TCT CGC-3'
Aluminum sulphate	Sigma-aldrich	368458	For Nuclear fast red solution preparation
Anti-Digoxigenin-AP, Fab fragments	Roche	11093274910	Antibody Registry ID: AB_514497
Anti-Human Natural Killer 1 antibody (HNK1, CD57)	Developmental Studies Hybridoma Bank	3H5	Antibody Registry ID: AB_2314644
Anti-mouse, goat IgM-HRP	Santa Cruz Biotechnology	sc-2973	Antibody Registry ID: AB_650513
Anti-mouse, goat IgG (H+L)-HRP	Novex	G-21040	Antibody Registry ID: AB_2536527
Anti-Smooth Muscle Actin/ACTA2 antibody	Dako	M085129	Antibody Registry ID: AB_2811108
Aquatex	Merck	1085620050	Aqueous mounting agent
5-Bromo-4-chloro-3-indolyl phosphate p-toluidine salt (BCIP)	Sigma-aldrich	B8503-100MG	
Blocking Reagent	Roche	11096176001	
Citric acid	VE TEC	238	For SSC buffer preparation
Collagenase type IA	Sigma-aldrich	SCR103	
dCTP, dGTP, dATP, dTTP set	Roche	11969064001	
Denhardt solution 50X	Invitrogen	750018	For hybridization buffer preparation
Dextran sulphate sodium salt	Thermo Scientific	15885118	For hybridization buffer preparation
DIG RNA Labeling Mix	Roche	11277073910	Contains Dig-11-dUTP

DMEM low-glucose	Sigma-aldrich	D5523	
3,3'-Diaminobenzidine tetrahydrochloride (DAB)	Sigma-aldrich	D5905-50TAB	
N,N-Dimethylformamide (DMF)	Sigma-aldrich	227056	For NBT and BCIP solution preparation
Ethylenediaminetetraacetic acid (EDTA)	Sigma-aldrich	E6758	For trypsin solution preparation
Entellan new	Merck	107961	Non-aqueous mounting medium
Ethanol	Proquímios	N/A	
Fetal bovine serum	ThermoFisher	12657029	Inactivate at 56 °C before use
Formaldehyde 37% solution	Proquímios	N/A	
Formamide	Vetec	V900064	
Glacial acetic acid	Proquímios	N/A	
India ink	Pelikan	221143	
L-glutamine solution (200 mM)	Gibco	25030-149	
Magnesium chloride	Merck	8147330100	For NTM buffer preparation
Maleic acid	Sigma-aldrich	M0375-500G	For MAB buffer preparation
Methanol	Proquímios		
Normal Goat Serum	Sigma-aldrich	NS02L	Inactivate at 56 °C before use
4-Nitro blue tetrazolium chloride (NBT)	Roche	11585029001	
Nuclear fast red	Sigma-aldrich	60700	
Paraplast Plus	Sigma-aldrich	P3558	
Penicillin G sodium salt	Sigma-aldrich	P3032	
Phosphate buffered saline (PBS)	Sigma-aldrich	P3813	
Phosphomolybdic acid	Merck	100532	
Proteinase K	Gibco BRL	25530-015	
Salmon sperm DNA	Invitrogen	15632011	For hybridization buffer preparation
Sodium chloride	Sigma-aldrich	S9888	For SSC, MAB and NTM buffer preparation
Streptomycin Sulfate	Sigma-aldrich	S6501	
Taq Polymerase kit	Cenbiot Enzimas	N/A	
Tris-HCl	Sigma-aldrich	T5941	
Trypsin	Sigma-aldrich	T4799	
Tween 20	Sigma-aldrich	P1379	
Xylene	Proquímios	N/A	
Microscope and equipments			
Axioplan upright microscope	Carl Zeiss Microscopy	N/A	
Axiovision software	Carl Zeiss Microscopy	N/A	
Cell incubator	ThermoForma	3110	
Egg incubator- 50 eggs	GP		
Gooseneck lamp	Biocam	N/A	For egg manipulation
Fiji software; Cell Counter plugin	ImageJ	https://imagej.net/software/fiji/	
Laminar flow hood	TROX	1385	
Nanodrop Lite	Thermo Scientific	ND-LITE-PR	
Rotary microtome	Leica Biosystems	RM2125 RTS	For sectioning
Stereomicroscope	Labomed	Luxeo 4D	For egg manipulation
Sterilization oven	REALIS	7261690	For sterilization of surgical materials
Consumables			

0.2 mL (PCR) polypropylene centrifuge tubes	Eppendorf	30124707	
15 mL polypropylene conical centrifuge tubes	Corning	CLS430791	
1.5 mL polypropylene centrifuge tubes	Axygen	MCT-150-C	
2 mL polypropylene centrifuge tubes	Axygen	MCT-200-C	
50 mL polypropylene conical centrifuge tubes	Corning	CLS430829	
Barrier (Filter) Tips, 200 µL size	Invitrogen	AM12655	For egg manipulation
Excavated Glass Block (Staining Block) with Cover Glass	Hecht Karl	42020010	
Embedding cassettes	Simport	M480	Used as a paraffin block holder
Glass coverslides, 24 x 40 mm	Kasvi	K5-2440	
Glass Pasteur pipettes 230 mm	NORMAX	5426023	For preparation of glass capillaries
Microtome blades	Leica Biosystems	HIGH-PROFILE-DISPOSABLE-BLADES-818	For sectioning
Parafilm M	Parafilm	P7793	
Plastic Petri dish, 30 mm	Kasvi	K13-0035	For egg manipulation
Plastic Petri dish, 60 mm	Prolab	0303-8	For cell spheroids preparation. Should not be treated for cell adhesion.
Silanized glass slides (Starfrost)	Knittel Glass	198	For sectioning
Syringe 1 mL , Needles 26 G (0.45 x 13 mm)	Descarpack	32972	For egg manipulation (albumen aspiration)
Surgical tools			
Aspirator tube	Drummond	2-000-000	For egg manipulation
Dissection scissors	Fine Science Tools	14061-11	For egg manipulation
Microforceps (tweezers)	Fine Science Tools	00108-11	For egg manipulation and preparation of glass capillaries
Needle holder (adjustable dissection needle chuck)	Fisherbrand	8955	For egg manipulation
Oil whetstone, 10.000 grit	N/A	N/A	For sharpening needles
Pair of small paint brushes	N/A	N/A	For handling paraffin sections. Any brand may be used.
Sewing needles	N/A	N/A	For sharpening into microscalpels. Any brand may be used.
Sterile disposable scalpel No. 23	Swann-Norton	110	For sectioning
Surgical scalpel handle	Swann-Norton	914	For sectioning
Wecker iris scissors, sharp/sharp	Surtex	SS-641-11	For egg manipulation