Materials List for:

Whole-mount Immunohistochemical Analysis for Embryonic Limb Skin Vasculature: a Model System to Study Vascular Branching Morphogenesis in Embryo

Wenling Li¹, Yoh-suke Mukouyama¹

¹Laboratory of Stem Cell and Neuro-Vascular Biology, Genetics and Developmental Biology Center, National Heart, Lung, and Blood Institute, National Institutes of Health

Correspondence to: Yoh-suke Mukouyama at mukoyamay@nhlbi.nih.gov

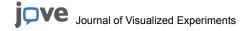
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Materials

Antibodies				
Pan-endothelial cel	l marker			
Antibody	Species	Company	Catalogue #	Working condition
PECAM-1	Armenian hamster (M)	Chemicon	MAB1398Z	1:100 dilution ^{#1}
PECAM-1	Rat (M)	BD	553369	1:300 dilution
VEGFR2	Rat (M)	eBioscience	14-5821-82	1:200 dilution
CD34	Rat (M)	eBioscience	13-0341	1:300 dilution
Collagen IV	Rabbit (P)	AbD Serotec	2150-1470	1:300 dilution ^{#2}
Arterial endothelial	cell marker	•	•	
Antibody	Species	Company	Catalogue #	Working condition
Neuropilin1	Rabbit (P)	The Alex Kolodkin's lab ^{#3}		1:3000 dilution
Unc5H2	Goat (P)	R&D	AF1006	1:200 dilution
Venous endothelial	cell marker			,
Antibody	Species	Company	Catalogue #	Working condition
EphB4	Goat (P)	R&D	AF446	1:100 dilution
Lymphatic endothe	lial cell marker	•	•	·
Antibody	Species	Company	Catalogue #	Working condition
LYVE-1 ^{#4}	Rabbit (P)	Abcam	ab14917	1:200 dilution
LYVE-1 ^{#4}	Rat (M)	MBL	D225-3	1:200 dilution
Prox-1	Rabbit (P)	Chemicon	AB5475	1:1000 dilution
Prox-1	Goat (P)	R&D	AF2727	1:50 dilution
Neuropilin2	Rabbit (P)	Cell Signaling	3366	1;100 dilution
Podoplanin	Syrian hamster (M)	Hybridoma Bank	8.1.1	1:200 dilution
Smooth muscle cel	l/pericyte marker			
Antibody	Species	Company	Catalogue #	Working condition
αSMA-Cy3	Mouse (M) ^{#5}	Sigma	c-6198	1:500 dilution ^{#6}
NG2	Rabbit (P)	Chemicon	AB5320	1:200 dilution
SM22α	Rabbit (P)	Abcam	ab14106	1:200 dilution

Antibodies for GFP reporter



Antibody	Species	Company	Catalogue #	Working condition
GFP	Rabbit (P)	Invitrogen	A11122	1:300 dilution
GFP	Rat (M)	Nacalai tesque	04404-84	1:1000 dilution
GFP	Chick (P)	Chemicon	P42212	1:300 dilution

Antibodies for LacZ reporter

Antibody	Species	Company	Catalogue #	Working condition
β-gal	Rabbit (P)	MP Biomedical	55976	1:5000 dilution
β-gal	Goat (P)	AbD Serotec	4600-1409	1:500 dilution
β-gal	Chick (P)	Abcam	ab9361	1:200 dilution

Antibodies for peripheral axon

Antibody	Species	Company	Catalogue #	Working condition
2H3	Mouse (M) ^{#7}	Hybridoma Bank	2H3	1:200 dilution
Tuj1	Mouse (M) ^{#8}	Covance	MMS-435P	1:500 dilution
Peripherin	Rabbit (P)	Chemicon	AB1530	1:1000 dilution

Antibodies for migrating Schwann cells

BFABP Rabbit (P)	The Thomas Müller's lab ^{#9}	1:3000 dilution	
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- (P): polyclonal antibody, (M): monoclonal antibody
- #1: Goat anti-Armenian hamster-Cy3 (Jackson ImmunoResearch 127-165-160) antibody should be used as a secondary antibody.
- #2: The Collagen IV antibody can be used to detect blood vessels after in situ hybridization.
- #3: The Neuropilin1 antibody is kindly provided by the Alex Kolodkin's lab in the Johns Hopkins University. Sheep anti-human Neuropilin1 antibody is available in R&D (AF3870), although we have not tested it yet.
- #4: The LYVE-1 antibodies also detect a subset of macrophages in the embryonic skin.
- #5: The anti- α SMA antibody is mouse IgG2a monoclonal antibody.
- #6: The Cy3-conjugated αSMA antibody is incubated for 1 hour at room temperature together with secondary antibodies for other primary antibodies.
- #7: 2H3 antibody is mouse IgG1 monoclonal antibody against neurofilament.
- #8: Tuj1 antibody is mouse IgG2a monoclonal antibody against Neuron-specific class III beta-tubulin.
- #9: The BFABP (brain-specific fatty acid binding protein) antibody is kindly provided by the Thomas Müller's lab in Max-Delbrück-Center for Molecular Medicine.