

Materials List for:

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

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## Materials

<b>Antibodies</b>				
<b>Pan-endothelial cell marker</b>				
Antibody	Species	Company	Catalogue #	Working condition
PECAM-1	Armenian hamster (M)	Chemicon	MAB1398Z	1:100 dilution <sup>#1</sup>
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 <sup>#2</sup>
<b>Arterial endothelial cell marker</b>				
Antibody	Species	Company	Catalogue #	Working condition
Neuropilin1	Rabbit (P)	The Alex Kolodkin's lab <sup>#3</sup>		1:3000 dilution
Unc5H2	Goat (P)	R&D	AF1006	1:200 dilution
<b>Venous endothelial cell marker</b>				
Antibody	Species	Company	Catalogue #	Working condition
EphB4	Goat (P)	R&D	AF446	1:100 dilution
<b>Lymphatic endothelial cell marker</b>				
Antibody	Species	Company	Catalogue #	Working condition
LYVE-1 <sup>#4</sup>	Rabbit (P)	Abcam	ab14917	1:200 dilution
LYVE-1 <sup>#4</sup>	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
<b>Smooth muscle cell/pericyte marker</b>				
Antibody	Species	Company	Catalogue #	Working condition
αSMA-Cy3	Mouse (M) <sup>#5</sup>	Sigma	c-6198	1:500 dilution <sup>#6</sup>
NG2	Rabbit (P)	Chemicon	AB5320	1:200 dilution
SM22α	Rabbit (P)	Abcam	ab14106	1:200 dilution
<b>Antibodies for GFP reporter</b>				

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) <sup>#7</sup>	Hybridoma Bank	2H3	1:200 dilution
Tuj1	Mouse (M) <sup>#8</sup>	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 <sup>#9</sup>		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-αSMA 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.