

Materials List for:

# Isolation of Human Lymphatic Endothelial Cells by Multi-parameter Fluorescence-activated Cell Sorting

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## Materials

Name	Company	Catalog Number	Comments
DMEM	Life Technologies (Gibco)	11965-092	Used to collect tissue samples from the operating theatre.
EGM-2 MV Bullet Kit	Lonza	CC-3202	EGM-2 MV Bullet Kit contains 500 ml of EBM-2 media and human EGF, hydrocortisone, gentamycin (GA-1000), fetal bovine serum (FBS), VEGF, human FGF-b, R <sup>3</sup> -IGF-1 and ascorbic acid.
VEGF-C	R&D Systems	2179-VC-025	Complete endothelial cell media is supplemented with 50 ng/ml VEGF-C
Antibiotic-antimycotic solution (100x)	Life Technologies (Gibco)	15240-062	This solution contains 10,000 U/ml of penicillin, 10,000 mg/ml of streptomycin and 25 mg/ml of Fungizone antimycotic. Use at 1:100 dilution.
Fibronectin from human plasma	Sigma-Aldrich	F2006	Used at 10 mg/ml concentrations. It can be re-used for up to 1 month if kept at 4 °C and maintained sterile. Use 7 ml to coat 150 cm <sup>2</sup> flask.
StemPro Accutase Cell dissociation reagent	Life Technologies (Gibco)	A11105-01	StemPro®Accutase® is used at 0.05 ml per cm <sup>2</sup> to cover the entire surface area of the flask. Using lesser volumes may result in incomplete cell detachment.
0.4% Trypan Blue dye	Life Technologies (Gibco)	15250-061	Used as a viability stain.
Dispase II	Roche Applied Biosciences	4942078001	Used at 0.04%
Collagenase II	Worthington Lab	4176	Used at 0.25%
DNase I	Roche Applied Biosciences	11284932001	Used at 0.01%
70 mm nylon cell strainers	BD Falcon	352350	

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PE-conjugated VEGFR-3 clone 9D9F9, clone WM59	BioLegend	356204	Used at 1:50 dilution
PE-Cy7-conjugated CD34, clone 581	BioLegend	343516	Used at 1:200 dilution
APC-conjugated mouse anti human CD31, clone WM59	BioLegend	303116,	Used at 1:100 dilution
Alexa 488-conjugated rat anti human Podoplanin, clone NC-80.	BioLegend	337006	Used at 1:200 dilution

PE-conjugated mouse IgG1, k isotype, clone MOPC-21	BioLegend	400112	Used at 1:50 dilution
PE-Cy7-conjugated mouse IgG1, k isotype, clone MOPC-21	BioLegend	400126	Used at 1:200 dilution
APC-conjugated mouse IgG1, k isotype, clone MOPC-21	BioLegend	400120	Used at 1:100 dilution
Alexa 488-conjugated mouse IgG2a, k isotype, clone RATK2758	BioLegend	400525	Used at 1:200 dilution