

# Isolation of Human Primary Valve Cells for In vitro Disease Modeling

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## Materials

Name	Company	Catalog Number	Comments
0.45 µm filter	Thermo Scientific	7211345	Preparing plate with collagen coating
10 cm cell culture plate	Greiner Bio-One	664160	Cell culture/cell line expansion
10 mL serological pipet	Fisher	14955234	VEC/VIC isolation, cell culture, cell line expansion
1000 µL filter tips	VWR	76322-154	Cell culture/cell line expansion
10XL filter tips	VWR	76322-132	Cell culture/cell line expansion
15 mL conical tubes	Thermo Scientific	339650	Tissue storage, VIC/VEC isolation
16% paraformaldehyde aqueous solution	Electron Microscopy Sciences	15710S	Tissue and cell fixative
190 proof ethanol	Decon	2801	Disinfection
1x DPBS: no calcium, no magnesium	Gibco	14190250	Saline solution. VIC/VEC isolation
1x PBS	Fisher	BP2944100	Saline solution. Tissue preparation, VIC/VEC isolation
20 µL filter tips	VWR	76322-134	Cell culture/cell line expansion
200 proof ethanol	Decon	2701	Deparaffinizing tissue samples
2-propanol	Fisher	A416P 4	Making collagen coated plates
5 mL serological pipet	Fisher	14955233	VEC/VIC isolation, cell culture, cell line expansion
50 mL conical tubes	Thermo Scientific	339652	Tissue storage, VIC/VEC isolation
60 mm dish	GenClone	25-260	VEC isolation
6-well cell culture plate	Corning	3516	Cell culture/cell line expansion
Acetic acid, glacial	Fisher	BP2401 500	Making collagen coated plates
AlexaFluor 488 phalloidin	Invitrogen	A12379	Fluorescent f-actin counterstain
Belzer UW Cold Storage Transplant Solution	Bridge to Life	BUW0011L	Tissue storage solution
Bovine Serum Albumin, Fraction V - Fatty Acid Free 25g	Bioworld	220700233	VEC confirmation with CD31+ Dynabeads
Calponin 1 antibody	Abcam	ab46794	Primary antibody (VIC positive stain)

CD31 (PECAM-1) (89C2)	Cell Signaling	3528	Primary antibody (VEC positive stain)
CD31+ Dynabeads	Invitrogen	11155D	VEC confirmation with CD31+ Dynabeads
CDH5	Cell Signaling	2500	Primary antibody (VEC positive stain)
Cell strainer with 0.70 µm pores	Corning	431751	VIC isolation
Collagen 1, rat tail protein	Gibco	A1048301	Making collagen coated plates
Collagenase II	Worthington Biochemical Corporation	LS004176	Tissue digestion. Tissue preparation, VIC/VEC isolation
Conflikt Ready-to-use Disinfectant Spray	Decon	4101	Disinfection
Countess II Automated Cell Counter	Invitrogen	A27977	Automated cell counter
Countess II reusable slide coverslips	Invitrogen	2026h	Automated cell counter required slide cover
Coverslips	Fisher	125485E	Mounting valve samples
Cryogenic vials	Olympus Plastics	24-202	Freezing cells/tissue samples
Disinfecting Bleach with CLOROMAX - Concentrated Formula	Clorox	N/A	Disinfection
DMEM	Gibco	10569044	Growth media. VIC expansion
EBM - Endothelial Cell Medium, Basal Medium, Phenol Red free 500	Lonza Walkersville	CC3129	Growth media. VEC expansion
EGM-2 Endothelial Cell Medium-2 - 1 kit SingleQuot Kit	Lonza Walkersville	CC4176	Growth media supplement. VEC expansion
EVOS FL Microscope	Life Technologies	Model Number: AME3300	Fluorescent imaging
EVOS XL Microscope	Life Technologies	AMEX1000	Visualizing cells during cell line expansion
Fetal Bovine Serum - Premium Select	R&D Systems	S11550	VIC expansion
Fine scissors	Fine Science Tools	14088-10	Tissue preparation, VIC/VEC isolation
Fisherbrand Cell Scrapers	Fisher	08-100-241	VIC expansion
Fungizone	Gibco	15290-026	Antifungal: Tissue preparation, VIC/VEC isolation
Gentamicin	Gibco	15710-064	Antibiotic: Tissue preparation, VIC/VEC isolation
Glass slides	Globe Scientific Inc	1358L	mounting valve samples
Goat anti-Mouse 488	Invitrogen	A11001	Fluorescent secondary Antibody
Goat anti-Mouse 594	Invitrogen	A11005	Fluorescent secondary Antibody
Goat anti-Rabbit 488	Invitrogen	A11008	Fluorescent secondary Antibody
Goat anti-Rabbit 594	Invitrogen	A11012	Fluorescent secondary Antibody
Invitrogen Countess II FL Reusable Slide	Invitrogen	A25750	Automated cell counter required slide
Invitrogen NucBlue Fixed Cell ReadyProbes Reagent (DAPI)	Invitrogen	R37606	Fluorescent nucleus counterstain
LM-HyCryo-STEM - 2X Cryopreservation media for stem cells	HyClone Laboratories, Inc.	SR30002	Frozen cell storage
Mounting Medium	Fisher Chemical Permout	SP15-100	Mounting valve samples
Mr. Frosty freezing container	Nalgene	51000001	Container for controlled sample freezing
Mycoplasma-ExS Spray	PromoCell	PK-CC91-5051	Disinfection
Penicillin-Streptomycin	Gibco	15140163	Antibiotic. VIC expansion
Plasmocin	Invivogen	ANTMPT	Anti-mycoplasma. VIC/VEC isolation and expansion

SM22a antibody	Abcam	ab14106	Primary antibody (VIC positive stain)
Sstandard pattern scissors	Fine Science Tools	14001-14	Tissue preparation, VIC/VEC isolation
Sterile cotton swab	Puritan	25806 10WC	VEC isolation
Swingsette human tissue cassette	Simport Scientific	M515-2	Tissue embedding container
Taylor Forceps (17cm)	Fine Science Tools	11016-17	Tissue preparation, VIC/VEC isolation
Trypan Blue Solution, 0.4%	Gibco	15250061	cell counting solution
TrypLE Express Enzyme	Gibco	12604021	Splitting VIC/VECs
Von Kossa kit	Polysciences	246331	Staining paraffin sections of tissues for calcification
von Willebrand factor antibody	Abcam	ab68545	Primary antibody (VEC positive stain)
Xylenes	Fisher Chemical	X3S-4	Deparaffinizing tissue samples
$\alpha$ SMA antibody	Abcam	ab7817	Primary antibody (VIC positive stain)