

Materials List for:

# Isolation, Expansion, and Adipogenic Induction of CD34+CD31+ Endothelial Cells from Human Omental and Subcutaneous Adipose Tissue

Bronson A. Haynes<sup>1</sup>, Ryan W. Huyck<sup>1</sup>, Ashley J. James<sup>1</sup>, Meghan E. Carter<sup>1</sup>, Omnia U. Gaafar<sup>1</sup>, Marjorie Day<sup>1</sup>, Avennette Pinto<sup>1</sup>, Anca D. Dobrian<sup>1</sup>

<sup>1</sup>Department of Physiological Sciences, Eastern Virginia Medical School

Correspondence to: Anca D. Dobrian at [dobriaad@evms.edu](mailto:dobriaad@evms.edu)

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## Materials

Name	Company	Catalog Number	Comments
<b>Large Equipment</b>			
Biosafety Cabinet	Nuaire	nu-425-400	
Cell Culture Incubator	Thermo-Fisher Scientific	800 DH	
Water Bath	Forma Scientific	2568	Reciprocal Shaker
RT-PCR Machine	BIO-RAD	CFX96-C1000	
Electrophoresis Box	BIO-RAD	Mini PROTEAN 3 Cell	
Transblot Box	BIO-RAD	Mini Trans-Blot Cell	
Electrophoresis Power Supply	BIO-RAD	PowerPac Basic	
ELISA Reader	Molecular Devices	SpectraMax M5	
Blot Reader	LI-COR	Odyssey	Near Infrared
Refrigerated Centrifuge	Eppendorf	5810 R	
Tabletop Centrifuge	Eppendorf	MiniSpin Plus	
Fluorescent Microscope	Olympus	BX50	
Inverted Microscope	Nikon	TMS	
<b>KRBSS Buffer</b>			
HEPES	Research Products International	H75030	
Sodium bicarbonate	Sigma-Aldrich	792519	
Calcium chloride dihydrate	Sigma-Aldrich	C7902	
Potassium phosphate monobasic	Sigma-Aldrich	P5655	
Magnesium sulfate	Sigma-Aldrich	M2643	
Sodium chloride	Sigma-Aldrich	746398	

Sodium phosphate monobasic monohydrate	Sigma-Aldrich	S9638	
Potassium chloride	Sigma-Aldrich	P9333	
Glucose	Acros Organics	410950010	
Adenosine	Acros Organics	164040250	
Bovine Serum Albumin	GE Healthcare Bio-Sciences	SH30574.02	
Penicillin/Streptomycin	Thermo-Fisher Scientific	15070063	
<b>Tissue Digestion</b>			
20 mL Syringe	Global Medical	67-2020	
Nylon Mesh, 250 µm	Sefar	03-250/50	
Pipetting Needles	Popper	7934	
Fine Scissors	Fine Science Tools	14058-11	
Tissue Forceps	George Tiemann & Co	160-20	
Collagenase, Type I	Worthington Biochemical	LS004196	
Petri Dishes, 100 mm	USA Scientific	5666-4160	TC Treated
Eppendorf Tubes, 1.5 mL	USA Scientific	1615-5500	
Conical Tubes, 15 mL	Nest Scientific	601052	
Conical Tubes, 50 mL	Nalgene	3119-0050	
Scintillation Vials	Kimble	74505-20	Tissue Dicing
<b>Cell Isolation</b>			
Cellometer	Nexcelom	Auto 2000	
Cellometer Slides	Nexcelom	CHT4-SD100-002	
Cellometer Viability Stain	Nexcelom	CS2-0106-5mL	Acridine Orange/Propidium Iodine
Anti-CD34 Magnetic Beads	StemCell Technologies	18056	Kit
EasySep Magnet	StemCell Technologies	18000	
Anti-CD31 Plastic Beads	pluriSelect USA	19-03100-10	
pluriSelect 10x Wash Buffer	pluriSelect USA	60-00080-10	
pluriSelect Connector Ring	pluriSelect USA	41-50000-03	
pluriSelect Detachment Buffer	pluriSelect USA	60-00046-12	
pluriSelect Incubation Buffer	pluriSelect USA	60-00060-12	

pluriSelect S Cell Strainer	pluriSelect USA	43-50030-03	
<b>Cell Culture</b>			
6-well Plates	USA Scientific	CC7682-7506	TC Treated
4-well chambered slides	Corning Life Sciences	354559	Fibronectin coated
4-well chambered slides	Thermo-Fisher Scientific	154526PK	Uncoated glass
Human Adipose Microvascular Endothelial Cells (HAMVEC)	ScienCell Research Laboratories	7200	Primary cell line
Endothelial Cell Media (ECM)	ScienCell Research Laboratories	1001	Complete Kit
DMEM/F12 Basal Media	Thermo-Fisher Scientific	11320082	
Fetal Bovine Serum (FBS)	Rocky Mountain Biologicals	FBS-BBT	
Insulin	Lilly	U-100	Humalog
Rosiglitazone	Sigma-Aldrich	R2408	
<b>Cell Analysis</b>			
Oil Red O Dye	Sigma-Aldrich	O0625	Prepared in isopropanol
96 well plates	USA Scientific	1837-9600	
96 well PCR plates	Genesee Scientific	24-300	
RNA Extraction	Zymo Research	R2072	Kit
cDNA Synthesis	BIO-RAD	1708841	Supermix
JumpStart PCR Polymerase	Sigma-Aldrich	D9307-250UN	Hot start, with PCR Buffer N
Magnesium Chloride Solution	Sigma-Aldrich	M8787-5ML	3 mM final in PCR reaction
dNTPs	Promega	U1515	
TaqMan AdipoQ	Thermo-Fisher Scientific	Hs00605917_m1	
TaqMan CIDEA	Thermo-Fisher Scientific	Hs00154455_m1	
TaqMan RPL27	Thermo-Fisher Scientific	Hs03044961_g1	
TaqMan UCP1	Thermo-Fisher Scientific	Hs00222453_m1	
BCA Assay	Sigma-Aldrich	QPBCA-1KT	Kit
Bis-acrylamide	BIO-RAD	1610146	40% stock solution
Ammonium Persulfate	BIO-RAD	1610700	
TEMED	BIO-RAD	1610800	
Tris	Sigma-Aldrich	T1503	

Glycine	BIO-RAD	1610718	
Sodium Dodecal Sulfate	Sigma-Aldrich	L3771	
EDTA	Fisher Scientific	S311-100	
Bromophenol Blue	Sigma-Aldrich	B8026	
Blot Membrane	EMD Millipore	IPFL00010	
Methanol	Fisher Scientific	A452-SK4	
Odyssey Blocking Buffer, Tris	LI-COR	927-50000	
Anti-AKT antibody	Cell Signaling Technology	2920S	Mouse monoclonal
Anti-pAKT antibody	Cell Signaling Technology	9271S	Rabbit polyclonal
Anti-UCP1 antibody	Abcam	ab10983	Rabbit polyclonal
Anti-Mouse IgG antibody	LI-COR	926-68070	Goat Polyclonal, IRDye 680RD
Anti-Rabbit IgG antibody	LI-COR	926-32211	Goat Polyclonal, IRDye 800CW
Anti-Rabbit IgG antibody	Jackson ImmunoResearch	111-025-003	Goat Polyclonal, TRITC
Phosphate Buffer Saline	Thermo-Fisher Scientific	10010049	
37% Formaldehyde Solution	Electron Microscopy Sciences	15686	4% solution for cell fixation
Normal Goat Serum	Vector Laboratories	S-1000	10% blocking solution
Triton X-100	Sigma-Aldrich	X-100	0.1% permeabilization solution
DAPI	Thermo-Fisher Scientific	D1306	
Calcein AM	Thermo-Fisher Scientific	65-0853-39	Cell fluorescent visualization
Matrigel Basement Membrane Matrix	Corning Life Sciences	356231	Growth factor reduced
Dil labeled Acetylated LDL	Thermo-Fisher Scientific	L3484	