

Materials List for:

Development and Identification of a Novel Subpopulation of Human Neutrophil-derived Giant Phagocytes *In Vitro*

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Materials

Name	Company	Catalog Number	Comments
Sterile scalp vein set (21GX3/4)	Bio Diagnostics Ltd.	# 20080312	A sterile needle for venipuncture
VACUETTE HOLDEX Single-Use Holder PP	Greiner Bio-One	# 450263	For securing during venipuncture
VACUETTE Tube K3E K3EDTA (16 x 100/9 mL)	Greiner Bio-One	# 455036	Sterile tube for blood collection
Nunclon MultiDish (24 well x 1 ml)	Thermo Scientific	# 142475	
Polypropylene conical centrifuge tube (50 ml)	Greiner Bio-One	# E14103PJ	
Transwell-24 well (transmigration assay)	Corning	# CA-3415	Polycarbonate membrane, 6.5 mm diameter, 3 µm pores)
RPML-1640 medium	BioIndustries	# 01-100-1A	Do not add antibiotics
EA.hy926 (ATCC CRL2922)	BioIndustries	# CRL-2922	
ATCC-formulated Dulbecco's Modified Eagle's Medium	BioIndustries	# 302002	complete growth medium
Polysucrose - Histopaque1119	Sigma-Aldrich	# 1119-1	Tissue culture grade
Polysucrose - Histopaque1077	Sigma-Aldrich	# 1077-1	Tissue culture grade
Phosphate buffered saline (PBS) - ion free	BioIndustries	# 02-023-1A	Cell biology and molecular biology grade
Heat inactivated Fetal calf serum (HI-FCS)	BioIndustries	# 04-121-1B	Cell biology grade or tissue culture grade, low LPS
NaCl	Sigma	# S3014	Molecular biology grade, suitable for cell culture
Paraformaldehyde, 16%	Electron Microscopy Sciences	# 15710	Cell biology grade or tissue culture grade (only 16% PFA)
Triton X-100	Sigma-Aldrich	# 9002-93-1	Molecular biology grade
Normal Goat Serum	BioIndustries	# 04-009-1	Cell biology and molecular biology grade
Trypan blue	BioIndustries	# 031021B	Tissue culture grade
May-Grünwald	Sigma-Aldrich	# MG500	Cell biology grade-(procedure No GS-10)
Giemsa stain Kit	Sigma	# 48900	Cell biology grade-(procedure No GS-10)
Fibronectin	BioIndustries	# 03090105	
Human Interleukin-8 (CXCL8)	PeproTech	# 200-08-5	
Anti-CD14 (clone 5A3B11B5)	Santa Cruz Biotechnologies	# sc-58951	Mouse IgG2b; expressed by monocytes
Anti-CD63 (clone MX-49.129.5)	Santa Cruz Biotechnologies	# sc-5275	Mouse IgG1; expressed by neutrophils

Anti-CD66b (clone 80H3)	AbD Serotec	# MCA216	Mouse IgG1; expressed by neutrophils
Anti-CD1c (BDCA-1) (clone AD5-8E7)	MACS Miltenyi Biotec	# 130-090-695	Mouse IgG2a; expressed by dendritic cells
Anti-CD15 (clone MY-1)	Abcam	# ab754	Mouse IgM; expressed by neutrophils
Anti-Cytochrome b-245 Light Chain (p22-phox) (clone 44.1)	BioLegend	# 650001	Mouse IgG2a; to recognize neutrophil NADPH oxidase complex
Anti-CD68	Protein Tech	# 16192-1-AP	Rabbit IgG; to recognize oxLDL scavenger receptor
Anti-LC3B	Sigma	# L7543	Rabbit IgG
Anti-Myeloperoxidase	Abcam	# ab45977	Rabbit IgG
Anti-Neutrophil elastase	Calbiochem	# 481001	Rabbit IgG
Anti-NOX2 (gp91-phox)	Abcam	# ab131083	Rabbit IgG
Anti-CD36 (SR-B3)	Novus Biologicals	# NB400-144	Rabbit IgG
Purified Mouse IgG1, κ Isotype Control (clone MG1-45)	BioLegend	# 401401	Antibody used as isotype control
Purified Mouse IgG2a, κ Isotype Control (clone MOPC-173)	BioLegend	# 400263	Antibody used as isotype control
Normal rabbit IgG	Santa Cruz Biotechnologies	# sc-2027	Antibody used as isotype control
CF488A Goat Anti-Rabbit IgG (H +L)	Biotium	# 20012	Anti-Rabbit IgG with the green fluorescent dye CF488A
CF647 Goat Anti-Rabbit IgG (H+L)	Biotium	# 20043	Anti-Rabbit IgG with the red fluorescent dye CF647
CF488A Goat Anti-Mouse IgG (H +L)	Biotium	# 20010	Anti-Mouse IgG with the green fluorescent dye CF488A
CF647 Goat Anti-Mouse IgG (H+L)	Biotium	# 20040	Anti-Mouse IgG with the red fluorescent dye CF647
Fluorescent Mounting Medium with DAPI	Vectashield H-1000; Vector Lab Inc.	# E19-18	Nuclear staining
Confocal laser scanning microscope (LSM 700)	Carl Zeiss	Ser.# 3523000380	Plan Apo 40X immersion oil objective
Zeiss CLSM software (ZEN 2010)	Carl Zeiss MicroImaging GmbH	version 6.0	For colocalization analysis
ImageJ software	Wayne Rasband, NIH, USA	version 1.49k	For determination of cell areas and fluorescence intensity
Light microscope (Axiovert 25)	Carl Zeiss	Ser.# 201060153	Examination of cells in culture
Centrifuge (Megafuge 1.0 R)	Heraeus Instruments	# D-37520	Cells separation from blood; cytopins preparation
Inverted fluorescent microscope (Zeiss Axio Observer Z.1)	Carl Zeiss	Ser.# 3834001470	Demonstration of giant phagocytes development
Temperature-controlled incubation system (Cube&Box)	Life Imaging Services		Temperature control system for microscopes
High resolution digital CCD camera (AxioCam HRm)	Carl Zeiss	Ser.# 117090279	For capturing high-contrast image data from an examined cell objects
Hydrophobic barrier pen (Elite PAP Pen)	Diagnostic Biosystems	# K039	For defining cell perimeter for immunofluorescence staining