

Materials List for:

# An Endothelial Planar Cell Model for Imaging Immunological Synapse Dynamics

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## Materials

Name	Company	Catalog Number	Comments
BD Vacutainer stretch latex free tourniquet	BD Biosciences	367203	
BD alcohol swabs	BD Biosciences	326895	
BD Vacutainer Safety-Lok	BD Biosciences	367861	K2 EDTA
BD Vacutainer Push Button Blood Collection Set	BD Biosciences	367335	
RPMI-1640	Sigma-Aldrich	R8758-1L	
Ficoll-Paque	Sigma-Aldrich	GE17-1440-02	Bring to RT before use
FCS-Optima	Atlanta Biologics	s12450	Heat inactivated
Penicillin-Streptomycin	Sigma-Aldrich	P4458-100ML	
Trypan blue	Sigma-Aldrich	T8154-20ML	
staphylococcal enterotoxin B	Toxin Technology	BT202RED	Stock solution 1mg/ml in PBS
toxic shock syndrome toxin 1	Toxin Technology	TT606RED	Stock solution 1mg/ml in PBS
human IL-15	R&D Systems	247-IL-025	Stock solution 50ug/ml in PBS
PBS	Life Technologies	10010-049	
Fibronectin	Life Technologies	33016-015	Stock solution 1mg/ml in H2O
HMVEC-d Ad-Dermal MV Endo Cells	Lonza	CC-2543	Other Human Microvascular ECs can be used, i.e. HLMVECs
EGM-2 MV bullet kit	Lonza	CC-3202	
Trypsin-EDTA	Sigma-Aldrich	T-4174	Stock solution 10x, dilute in PBS
amaxa-HMVEC-L Nucleofector Kit	Lonza	vpb1003	Required Kit for step 4
IFN-g	Sigma-Aldrich	I3265	Stock solution 1mg/ml in H2O
TNF-alpha 10ug, human	Life Technologies	PHC3015	Stock solution 1mg/ml in H2O
phenol Red-free HBSS	Life Technologies	14175-103	
Hepes	Fisher Scientific	BP299-100	
Calcium Chloride	Sigma-Aldrich	C1016-100G	Stock solution 1M in H2O
Magnesium chloride	Sigma-Aldrich	208337	Stock solution 1M in H2O
Human Serum albumin	Sigma-Aldrich	A6909-10ml	
Immersol 518 F fluorescence free Immersion oil	Fisher Scientific	12-624-66A	
Fura-2 AM 20x50ug	Life Technologies	F1221	Stock solution 1mM in DMSO
pEYFP-Mem (Mem-YFP)	Clontech	6917-1	
pDsRed-Monomer (Soluble Cytoplasmic DsRed)	Clontech	632466	
pDsRed-Monomer Membrane (Mem-DsRed)	Clontech	632512	

pEGFP-Actin	Clontech	6116-1	
Alexa Fluor 488 Phalloidin	Life Technologies	A12379	
Formaldehyde solution 37%	Fisher Scientific	BP531-500	Toxic, use fumehood
Triton X-100	Sigma-Aldrich	X100-5ML	
Falcon 15mL Conical Centrifuge Tubes	Fisher Scientific	14-959-70C	
Falcon 50mL Conical Centrifuge Tubes	Fisher Scientific	14-959-49A	
Falcon Tissue Culture Treated Flasks T25	Fisher Scientific	10-126-9	
Falcon Tissue Culture Treated Flasks T75	Fisher Scientific	13-680-65	
Corning Cell Culture Treated T175	Fisher Scientific	10-126-61	
Glass coverslips	Fisher Scientific	12-545-85	12 mm diameter
Falcon Tissue Culture Plates 24-well	Fisher Scientific	08-772-1	
Delta-T plates	Bioptechs	04200415B	
Wheaton Disposable Pasteur Pipets	Fisher Scientific	13-678-8D	
1.5 ml Eppendorf tube	Fisher Scientific	05-402-25	
ICAM1 mouse anti-human	BD Biosciences	555509	
HS1 mouse anti-human	BD Biosciences	610541	
Anti-Human CD11a (LFA-1alpha) Purified	ebioscience	BMS102	
Anti-Human CD3 Alexa Fluor® 488	ebioscience	53-0037-41	
Anti-MHC Class II antibody	Abcam	ab55152	
Anti-Talin 1 antibody	Abcam	ab71333	
Anti-PKC theta antibody	Abcam	ab109481	
phosphotyrosine (4G10 Platinum)	Millipore	50-171-463	
Nucleofector II	Amaxa Biosystems		Required electroporator for step 4
Zeiss Axiovert	Carl Zeiss MicroImaging		
Zeiss LSM510	Carl Zeiss MicroImaging		
Zeiss Axiovision Software	Carl Zeiss MicroImaging		
NU-425 (Series 60) Biological Safety Cabinet	NuAIRE	Nu-425-600	
Forma STRCYCLE 37 °C, 5% CO2 Cell culture Incubator	Fisher Scientific	202370	
Centrifuge 5810	Eppendorf	EW-02570-02	
Hemocytometer	Sigma-Aldrich	Z359629	Bright-Line Hemocytometer
Isotemp Waterbath model 202	Fisher Scientific	15-462-2	