

Materials List for:

Analyzing the Effects of Stromal Cells on the Recruitment of Leukocytes from Flow

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Materials

Name	Company	Catalog Number	Comments
Collagenase Type Ia	Sigma	C2674	Dilute in 10 ml PBS to get a final concentration of 10 mg/ml. Store at -20 °C in 1 ml aliquots.
Dulbecco's PBS	Sigma	D8662	With calcium and magnesium chloride. Keep sterile and store at RT.
1X Medium M199	Gibco	31150-022	Warm in 37 °C water bath before use.
Gentamicin sulphate	Sigma	G1397	Store at 4 °C. Add to M199 500 ml bottle.
Human epidermal growth factor	Sigma	E9644	Store at -20 °C in 10 µl aliquots.
Fetal calf serum (FCS)	Sigma	F9665	FCS must be batch tested to ensure the growth and viability of isolated EC. Heat inactivate at 56 °C. Store in 10 ml aliquots at -20 °C.
Amphotericin B	Gibco	15290-026	Potent and becomes toxic within a week so fresh complete HUVEC medium must be made up every week. Store at -20 °C in 1 ml aliquots.
Hydrocortisone	Sigma	H0135	Stock is in ethanol. Store at -20 °C in 10 µl aliquots.
Collagenase Type II	Sigma	C6885	Dilute stock in PBS to a final concentration of 100 mg/ml. Store at -20 °C in 100 µl aliquots.
Hyaluronidase	Sigma	H3631	Dilute stock in PBS to a final concentration of 20,000 U/ml. Store at -20 °C in 100 µl aliquots.
100 µm cell strainer for 50 ml centrifuge tube	Scientific Lab Supplies (SLS)	352360	Other commercially available cell strainers (e.g. Greiner bio-one) can also be used.
DMEM low glucose	Biosera	LM-D1102/500	Warm in 37 °C water bath before use.
Penicillin/Streptomycin mix	Sigma	P4333	Store at -20 °C in 1 ml aliquots.
25 cc tissue culture flask	SLS	353109	
75 cc tissue culture flask	SLS	353136	
Bone marrow mesenchymal stem cells vial	Lonza	PT-2501	Store in liquid nitrogen upon arrival. Cells are at passage 2 upon arrival but are designated passage 0. Expand to passage 3

			and store in liquid nitrogen for later use.
Mesenchymal stem cell growth medium (MSCGM)	Lonza	PT-3001	Warm in 37 °C water bath before use. For Cell Tracker Green staining use medium without FCS.
EDTA (0.02%) solution	Sigma	E8008	Store at 4 °C. Warm in 37 °C water bath before use.
Trypsin solution	Sigma	T4424	Store at -20 °C in 2 ml aliquots. Thaw at RT and use immediately.
Cryovials	Greiner bio-one	2019-02	Keep on ice before adding before adding cell suspension.
Mr. Frosty Freezing Container	Nalgene	5100-0001	Store at RT. When adding cryovials with cells store at -80 °C for 24 hr before transferring cells to liquid nitrogen.
Ibidi u-Slide VI (0.4), T/C treated, sterile	Ibidi	IB-80606	Alternative models include glass capillaries, Cellix Biochips (www.cellixltd.com), BioFlux Plates (www.fluxionbio.com/bioflux/) and GlycoTech parallel plate flow chambers (http://www.glycotech.com/apparatus/parallel.html).
Cell tracker green dye	Life technologies	C2925	Store in 5 µl aliquots at -20 °C. Dilute in 5 ml prewarmed (at 37 °C) MSCGM.
Cell counting chambers	Nexcelom	SD-100	Alternatively a haemocytometer can be used.
Cellometer auto T4 cell counter	Nexcelom	Auto T4-203-0238	
Tumor necrosis factor α (TNFα)	R&D Systems	210-TA-100	Dilute stock in PBS to a final concentration of 100,000 U/ml. Store at -80 °C in 10 µl aliquots.
6-well, 0.4 µm PET Transwell filters	SLS	353090	
K2-EDTA in 10ml tubes	Sarstedt		Store at RT.
Histopaque 1119	Sigma	11191	Store at 4 °C. Warm to RT before use.
Histopaque 1077	Sigma	10771	Store at 4 °C. Warm to RT before use.
10 ml round bottomed tube	Appleton Woods	SC211 142 AS	
7.5% BSA Fraction V solution	Life technologies	15260-037	Store at 4 °C.
20 ml Plastipak syringes	BD falcon	300613	
5 ml Plastipak syringes	BD falcon	302187	
2 ml Plastipak syringes	BD falcon	300185	
3M hypo-allergenic surgical tape 9 m x 2.5 cm	Micropore	1530-1	Use to secure the syringe tap onto the wall of the perspex chamber.
Silicon rubber tubing, internal diameter/external diameter (ID/OD) of 1/3 mm (thin tubing)	Fisher Scientific	FB68854	Cut silicon tubing to the appropriate size. All tubing leading directly to the electronic microvalve must be thin.
Silicon rubber tubing ID/OD of 2/4 mm (thick tubing)	Fisher Scientific	FB68855	
Portex Blue Line Manometer tubing	Smiths	200/495/200	Tubing leading to the syringe pump.
3-way stopcock	BOC Ohmeda AB		
Glass 50 ml syringe for pump	Popper Micromate	550962	Must be primed prior to use by removing any air bubbles.

Glass coverslip	Raymond A Lamb		26 x 76 mm coverslips made to order. Lot number 2440980.
Parafilm gasket	American National Can Company		Cut a 26 x 76 mm piece of parafilm using an aluminium template and cut a 20 x 4 mm slot into it using a scalpel 10a. Gasket thickness is approximately 133 µm.
Two perspex parallel plates	Wolfson Applied Technology Laboratory		Specially designed chamber consisting of parallel plates held together by 8 screws. The lower plate has a viewing slot cut out in the middle and a shallow recess milled to allow space for the coverslip, filter and gasket. The upper perspex plate has an inlet and outlet hole positioned over the flow channel.
Electronic 3-way microvalve with min. dead space	Lee Products Ltd.	LFYA1226032H	Electronically connected to a 12 volt DC power supply.
Syringe pump for infusion/ withdrawal (PHD2000)	Harvard Apparatus	70-2001	Set the diameter to 29 mm and refill (flow) rate.
L-shaped connector	Labhut	LE876	To attach to the inlet and outlet ports onto the Ibidi microslide channel.
Video camera	Qimaging	01-QIC-F-M-12-C	Connected to a computer which enables digitall videos to be recorded.
Image-Pro Plus 7.0	Media Cybernetics	41N70000-61592	For data analysis. Manually tag cells displaying the different behaviors. Track cells for analysis of rolling and migration velocities.
Refer to product datasheets for details on hazards of using the reagents described here.			