

Materials List for:

***In vitro* Coculture Assay to Assess Pathogen Induced Neutrophil Trans-epithelial Migration**

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Materials

Name	Company	Catalog Number	Comments
NCI-H292 cells	ATCC	CRL-1848	
RPML-1640 medium	ATCC	30-2001	
<i>Pseudomonas aeruginosa</i> PAO1	ATCC	#47085	
<i>Escherichia coli</i> MC1000	ATCC	#39531	
D-PBS (1x) liquid	Invitrogen	14190-144	without calcium and magnesium
Heat Inactivated Fetal bovine serum	Invitrogen	10082-147	10% added to culture medium
Penicillin-Streptomycin	Invitrogen	15140-122	100x: 10,000 units of penicillin and 10,000 µg of streptomycin per ml.
Trypsin-EDTA (0.05%)	Invitrogen	25300-062	50 ml aliquots are stored frozen at -20 °C. Aliquot in use can be stored at 4 °C short-term.
Hank's Balanced Salt Solution - HBSS(-)	Invitrogen	14175-079	Sterile, without calcium and magnesium
Trypan Blue Solution	Invitrogen	15250-061.	Stock = 0.4%
Collagen, Rat Tail	Invitrogen	A10483-01	Can also be isolated in the laboratory directly from the tails of rats using standard protocols
Citric acid	Sigma-Aldrich	C1909-500G	Component of 1 M citrate buffer and acid citrate dextrose (ACD) solution
Sodium Citrate	Sigma-Aldrich	S4641-500G	Component of 1 M citrate buffer
Dextrose anhydrous	Sigma-Aldrich	D8066-250G	Component of acid citrate dextrose (ACD) solution
Ammonium Chloride	Sigma-Aldrich	213330-500G	Component of red blood cell (RBC) lysis buffer
Sodium bicarbonate	Sigma-Aldrich	S6014-500G	Component of red blood cell (RBC) lysis buffer
EDTA	Sigma-Aldrich	ED-100G	Component of red blood cell (RBC) lysis buffer
HBSS(+) powder	Sigma-Aldrich	H1387-10L	Key component of HBSS+
HEPES	Sigma-Aldrich	H3375-500G	Component of HBSS+
Sigmacote	Sigma-Aldrich	SL2-25ML	Follow vendor instructions to coat glass pipette tips
Triton X-100	Sigma-Aldrich	T-9284	
2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS)	Sigma-Aldrich	A9941-50TAB	Key component of ABTS substrate solution

30% Hydrogen Peroxide Solution	Sigma-Aldrich	H1009-100ML	Component of ABTS substrate solution
N-Formyl-Met-Leu-Phe (fMLP or fMLF)	Sigma-Aldrich	F-3506	A Stock solution of 10 mM in DMSO should be prepared and aliquots stored at -20 °C.
Gelatin Type B	Fisher Scientific	M-12026	
Pseudomonas isolation agar	Fisher Scientific	DF0927-17-1	Follow manufacturer's instructions to make PIA plates
Ficoll-Paque PLUS	Fisher Scientific	45-001-749	Optional, can improve neutrophil purity
24-well migration plate	Corning Incorporated	#3524	
24-well wash plate	Falcon	35-1147	Can be reused if soaked in 70% ethanol and washed thoroughly prior to reuse
96-well plate	Fisher Scientific	#12565501	
Transwell Permeable Supports	Corning Incorporated	#3415	Polycarbonate; Diameter: 6.5 mm; Growth area: 0.33 cm ² ; Dish style: 24-well plate; Pore size: 3.0 µm
Petri dish	Falcon	35-1013	Each Petri dish holds 24 inverted 0.33 cm ² Transwells.
500 ml 0.2 µm filter / flask	Fisher Scientific	09-740-25A	To sterilize acid citrate dextrose (ACD) solution
5-3/4 in glass Pasteur pipette	Fisher Scientific	13-678-20A	Coat tips with Sigmacote prior to use
Hemostat	Fisher Scientific	13-812-14	Curved, Serrated
Invertoskop Inverted Microscope	Zeiss	#342222	
Versa-Max Microplate Reader	Molecular Devices	#432789	