

Materials List for:

Implementation of a Permeable Membrane Insert-based Infection System to Study the Effects of Secreted Bacterial Toxins on Mammalian Host Cells

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Materials

Name	Company	Catalog Number	Comments
Todd-Hewitt broth	Acumedia	7161A	Use appropriate broth for bacterial species of interest.
BioSpectrometer	Eppendorf	basic model	Any UV-Vis spectrophotometer is suitable.
Petri Dishes	Fisher Scientific	FB0875713	Other brands are suitable.
Agar	Sigma	A1296-1kg	Other brands are suitable.
HaCaT human epithelial keratinocytes			Gift from lab of Victor Nizet.
Dulbecco's Modified Eagle's Medium (DMEM)	Life Technologies	11995-073	Use appropriate media for cell line of interest.
Fetal Bovine Serum (FBS)	Biowest	S162H	Use appropriate media additives for cell line of interest.
10 cm tissue culture dishes	Nunc	150350	Other brands are suitable.
6 well tissue culture dishes	CytoOne	cc7682-7506	Other brands are suitable but need to be compatible with the Corning insert.
24 well tissue culture dishes	CytoOne	cc7682-7524	Other brands are suitable but need to be compatible with the Corning insert.
Glass coverslips	Fisherbrand	12-541-B	These must be autoclaved prior to use for cell plating.
Phosphate Buffered Saline (PBS)	Gibco	10010-023	
Phenol Red Free DMEM	Life Technologies	31053028	Phenol Red Free media is only required for the LDH release assay.
Bovine Serum Albumin (BSA)	Fisher Scientific	BP1600-100	
L-glutamine	Gibco	25030149	Only required to supplement cell culture media for LDH release assay.
Sodium pyruvate	Gibco	BP356100	Only required to supplement cell culture media for LDH release assay.
Collagen-coated 0.4 µm Transwell inserts (6 well)	Corning	3540	
Collagen-coated 0.4 µm Transwell inserts (24 well)	Corning	3595	
Forceps	Fisher	3120018	These must be sterilized with ethanol prior to handling Transwell inserts.
Cell scrapers	Fisherbrand	08-100-241	These are only required for the collection of cell lysates.

Paraformaldehyde	Fisher Scientific	04042-500	TOXIC. This is only required for immunofluorescence imaging.
Bicinchoninic acid (BCA) assay kit	Pierce	23227	Other protein quantification assays may be used.
Tris-glycine 4 - 15% polyacrylamide gels	BioRad	4561083	Buffer system and percent polyacrylamide should be selected based on proteins of interest.
Electrophoresis cassette supplies	BioRad	1658063	Only required for Western Blotting.
Electrophoresis power supply	BioRad	1645050	Only required for Western Blotting.
Western blot transfer cassette	BioRad		Only required for Western Blotting.
Polyvinylidene (PVDF) Membrane	EMD Millipore	IPVH00010	Only required for Western Blotting.
Tween 20	Sigma	P1379-500ml	
Rabbit IgG-HRP secondary antibody	Santa Cruz Biotechnology	sc2030	The secondary antibody should be determined based on the selected method of detection.
Mouse IgG-HRP secondary antibody	Santa Cruz Biotechnology	sc2031	The secondary antibody should be determined based on the selected method of detection.
Phospho-p38 (T180 + T182) MAPK antibody	Cell Signaling	4511	Select appropriate primary antibodies based on proteins of interest.
Total p38 MAPK antibody	Cell Signaling	8690	Select appropriate primary antibodies based on proteins of interest.
Goat anti-rabbit IgG Alexafluor 488	Molecular Probes	A-11034	Other secondary antibodies may be used for immunofluorescence imaging detection.
LumiGLO Detection Reagent	KPL	54-61-00	Only required for Western Blotting with detection on film.
Detection film	Biodot	BDB57	Only required for Western Blotting with detection on film.
DeltaVision Nikon 90i fluorescence microscope	Nikon		Other fluorescence microscopes are suitable for these analyses.
Normal goat serum	Thermo Scientific	31873	
Triton X-100	Sigma	T9284-500mL	
DAPI nuclear stain	Cell Signaling	4083	Select stains based on specific immunofluorescence applications of interest.
Rhodamine-phalloidin actin stain	Molecular Probes	R415	Select stains based on specific immunofluorescence applications of interest.
Fluoromount-G	Southern Biotech	0100-01	Only required for immunofluorescence imaging.
Ethidium homodimer 1	Molecular Probes	E1169	Only required for membrane permeabilization cytotoxicity assay.
Spectramax M5 Microplate Reader	Molecular Devices		Other microplate readers capable of detecting UV-vis, fluorescence and luminescence may be suitable.
Saponin	Sigma	47036-50G-F	Only required for membrane permeabilization cytotoxicity assay.
Molecular Probes ATP Determination Kit	Life Technologies	A22066	Other kits are likely to be suitable.
Cytotoxicity Detection Kit for LDH release	Roche	11644793001	Other kits are likely to be suitable.
Nonidet P40 Substitute	Sigma	74385-1L	Other suppliers are suitable.

HALT Phosphatase Inhibitor Cocktail	Thermo Scientific	78420B	Other cocktails are likely to be suitable.
SIGMAFAST Protease Inhibitor Cocktail Tablets, EDTA-free	Sigma	S8830-2TAB	Other cocktails are likely to be suitable.