

Materials List for

Deciphering the Molecular Mechanism and Function of Pore-Forming Toxins using *Leishmania major*

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Materials

Name	Company	Catalog Number	Comments
1.2 mL microtiter (Marsh) tubes	Fisher	02-681-376	Cytotoxicity assay
1.5 mL microcentrifuge tube	Fisher	05-408-129	Toxin dilutions
15 mL centrifuge tube	Avantor VWR (Radnor, PA)	89039-666	To hold cells and media
1x Phosphate buffered saline (PBS)	Fisher	BP399	For cell processing
3% H ₂ O ₂	Walmart (Fayetteville, AR)	N/A	For ECL
5x M199	Cell-gro	11150067	Basal growth media for <i>L. major</i> promastigotes
Biosafety cabinet	Baker		To culture cells in sterile conditions
Bovine serum albumin (BSA)	Fisher	BP1605-100	Fraction V acceptable purity
CaCl ₂	Fisher	BP510-100	Stock concentration 100 mM
Centrifuge	Thermo Fisher	Heraeus Megafuge 40R	To pellet the cells from culture
Cy5 Mono-reactive dye pack	Cytiva (Marlborough, MA)	PA25031	Fluorophore label for toxins
Digital dry bath	Benchmark	BSH1002	To denature protein samples
EGTA	Amresco	0732-100G	Stock concentration 0.5 M
Excel	Microsoft (Redmond, VA)		Data analysis software
Flow cytometer (4-laser Attune NxT)	Fisher		Cytometer for data acquisition
FlowJo	BD (Ashland, OR)		Software
Formaldehyde	Fisher	BP531-500	Fixative for counting cells
G418	Fisher	BP673-1	Selection agent for cells
Hellmanex III	Sigma	Z805939	Dilute 1:4 for cleaning cytometer
Hemocytometer	Fisher	0267151B	For counting cells
Human red blood cells	Zen-bio (Durham, NC)	SER-10MLRBC	To validate toxin activity
Ice bucket			
Light microscope	Nikon	Eclipse 55i	To visualize cells
Nitrocellulose	Fisher	88018	For probing proteins via antibodies
Pipettors and tips	Avantor VWR		To dispense reagents
Power supply	Bio-Rad		To run SDS-PAGE and transfers

Propidium iodide	Biotium	40016	Stock concentration 2 mg/mL in water
Protein ladder	Bio-Rad	161-0373	To determine molecular weight of proteins
SDS-PAGE Running Apparatus (Mini Protean III)	Bio-Rad	165-3302	To separate proteins based on their size
Sealing tape	R&D	DY992	To seal plates with cells
Streptolysin O C530A plasmid insert			Cloned into pBAD-gIII vector (Reference: 7)
Streptolysin O C530A toxin	Lab purified		Specific activity 4.34×10^5 HU/mg
Swinging bucket rotor	Thermo Fisher	75003607	To centrifuge cells
V-bottom plate	Greiner Bio-one	651206	For cytotoxicity assay
Vortex	Benchmark	BV1000	To mix cells
Western blot imaging system (Chemi-doc)	Bio-Rad		To visualize proteins by western blot
Western Blot Transfer Apparatus (Mini Protean III)	Bio-Rad	170-3930	Transfer proteins to nitrocellulose
Whatman Filter paper	GE Healthcare Life Sciences	3030-700	Used in transfer of proteins to nitrocellulose
Antibody			
Anti-ERK antibody	Cell Signaling Technologies	Cat# 9102S	Rabbit (1:1000 dilution)
Anti-lipophosphoglycan (LPG) antibody	CreativeBioLabs	Cat# WIC79.3	Mouse (1: 1000)
Anti-MEK antibody	Cell Signaling Technologies	Cat# 9122L	Rabbit (1:1000)
Anti-mouse IgG, HRP conjugate	Jackson ImmunoResearch	Cat#715-035-151	Donkey (1:10000)
Anti-phosphoERK antibody	Cell Signaling Technologies	Cat# 9101S	Rabbit (1:1000)
Anti-pMEK antibody	Cell Signaling Technologies	Cat# 9121S	Rabbit (1:1000)
Anti-rabbit IgG, HRP conjugate	Jackson ImmunoResearch	Cat#711-035-152	Donkey (1:10000)
Anti-tubulin antibody	Sigma	Cat# T5168	Mouse (1: 2000)
Leishmania major Genotypes			
Episomal addback (<i>spt2⁺</i> /+SPT2)			Δ spt2::HYG/ Δ spt2::PAC/+pXG-SPT2
Serine palmitoyltransferase subunit 2 knockout (<i>spt2⁻</i>)			Δ spt2::HYG/ Δ spt2::PAC
Wild type (WT)			LV39 clone 5 (Rho/SU/59/P)