

Materials List for:

# Macrophage Cholesterol Depletion and Its Effect on the Phagocytosis of *Cryptococcus neoformans*

Arielle M. Bryan<sup>\*1</sup>, Amir M. Farnoud<sup>\*1</sup>, Visesto Mor<sup>1</sup>, Maurizio Del Poeta<sup>1</sup>

<sup>1</sup>Department of Molecular Genetics and Microbiology, Stony Brook University

\*These authors contributed equally

Correspondence to: Maurizio Del Poeta at [maurizio.delpoeta@stonybrook.edu](mailto:maurizio.delpoeta@stonybrook.edu)

URL: <https://www.jove.com/video/52432>

DOI: [doi:10.3791/52432](https://doi.org/10.3791/52432)

## Materials

Name	Company	Catalog Number	Comments
Class II type A2 Biosafety Cabinet	Labconco	3460009	
J774A.1 cell line	ATCC	TIB-67	Arrives Frozen. See ATCC instructions for culturing.
Dulbecco's Modified Eagle Medium	Gibco	11995-065	Store at 4 °C and warm to 37 °C prior to use
HI Fetal Bovine Serum Performance Plus	Gibco	10082-147	Keep frozen at -20 °C and thaw before adding to DMEM
Penicillin-Streptomycin (10,000 U/ml)	Gibco	15140-122	Used to supplement DMEM
Isotemp Cell culture incubator	Fisher Scientific		Model # 3530
96-Well culture dish	Corning Inc. Costar	3595	
10x Phosphate Buffered Saline	Fisher Scientific BioReagents	BP3994	Dilute to 1x and filter or autoclave prior to use.
Methyl-β-cyclodextrin	Sigma Life Science	C4555-10G	Dissolve in 1x PBS to make solutions of 10 mM and 30 mM concentrations
Orbital shaker	Labline		
Amplex Red Cholesterol Assay Kit	Life Technologies Molecular Probes	A12216	All reagents for Cholesterol Assay are contained within the kit. Follow Manufacturer instructions.
96-Well Black Assay plate	Corning Inc. Costar	3603	
FilterMax microplate reader	Molecular Devices		Model F5
TLC chamber	Sigma-Aldrich	Z126195-1EA	
Chloroform	Sigma-Aldrich	650498-4L	
Methanol	Sigma-Aldrich	34860-2L-R	
TLC Paper	Whatman	3030917	Cut down to size needed for TLC tank
Fume hood			Any fume hood that complies with AIHA/ANSI Standards
6-Well plate	Corning Inc. Costar	3506	
Trypsin-EDTA	Gibco	25300-054	
Cell scraper	Corning Inc. Costar	3010	
Hemocytometer	Hausser Scientific	1490	
Centrifuge	Beckman Coulter		Model Alegria x-30R
Vortex mixer	Fisher Scientific	12-812	
Balance	Mettler Toledo		Model # MS104S measures down to 0.1 mg

Glass Pasteur pipette	Fisherbrand	13-678-20A	
Cholesterol	Avanti Polar Lipids	700000	
SpeedVac concentrator	Thermo Scientific		Model # SPD2010
Petroleum ether	Fisher Scientific	E139-1	
Diethyl ether	Sigma-Aldrich	309966	
Acetic acid	Sigma-Aldrich	320099	
TLC Silica Gel 60 with concentrating zone	Analytical Chromatography Millipore	1.11845.0001	
Iodine chips	Sigma-Aldrich	376558-50G	
Sulfuric acid	Sigma-Aldrich	320501	
Manganese chloride	Sigma-Aldrich	244589	
UVP EC3 Imaging System	Ultra-Violet Products Ltd.		Use the Vision Works LS software for densitometry analysis
Glass bottom confocal dish	MatTek	P35G-1.5-10C	www.glassbottomdishes.com
Cryptococcus neoformans (H99)			Obtained from Duke University Medical Center
YNB	BD	239210	See manufacturer for preparation instructions. Use a glucose concentration of 20 g/L.
Lipopolysaccharide	Sigma	L4391-1MG	Dissolve in 1x PBS to make 1 mg/ml stock. Store at -20 °C.
Interferon gamma	Sigma	I4777	Dissolve in 1x PBS to make 0.1 mg/ml stock solution
Glucuronoxylomannan antibody (anti-GXM)			Gift from Arturo Casadevall's Lab concentration is 1.98 mg/ml
Giemsa	MP Biomedicals	194591	Dissolve 0.8 g of Giemsa in 25 ml of glycerol and heat to 60 °C for 1 hr. Add 25 ml of methanol to the solution and allow to age at room temperature for at least 1 month.
Microscope	Zeiss		Observer.D1 microscope with AxioCam MRm for taking images