

Materials List for:

Size Matters: Measurement of Capsule Diameter in *Cryptococcus neoformans*

Tiffany Guess¹, Hoyin Lai², Serenah E. Smith¹, Linda Sircy¹, Kirsten Cunningham¹, David E. Nelson^{*1}, Erin E. McClelland^{*1}

¹Department of Biology, Middle Tennessee State University

²DRVision Technologies

*These authors contributed equally

Correspondence to: David E. Nelson at david.e.nelson@mtsu.edu, Erin E. McClelland at Erin.McClelland@mtsu.edu

URL: <https://www.jove.com/video/57171>

DOI: [doi:10.3791/57171](https://doi.org/10.3791/57171)

Materials

Name	Company	Catalog Number	Comments
Capsule Induction			
<i>C. neoformans</i> cells			The clinical lab strain, H99S, was a kind gift from Dr. John Perfect (Duke University). The clinical strains, B18 and B52, were kind gifts from Dr. Greg Bisson (University of Pennsylvania).
Yeast Peptone Dextrose Broth (YPD)	Fisher Scientific	DF0428-17-5	
Phosphate Buffered Saline (PBS)			This is made in the lab using standard recipe (137mM NaCl, 2.7 mM KCl, 10mM Na ₂ HPO ₄ O, 2 mM K ₂ HPO ₄ O)
DMEM/high-glucose with L-glutamine, without sodium pyruvate	GE Life Sciences	SH30022.01	
6-well plates	Falcon	CL5335-5EA	
Shaking incubator	Thermo Scientific		MaxQ6000
CO ₂ incubator	Fisher Scientific		Isotemp
Centrifuge	Thermo Scientific		Legend XTR
Staining			
Microcentrifuge	Thermo Scientific		Legend Micro 21R
India ink	Fisher Scientific	14-910-56	
Calcofluor white	Sigma-Aldrich	18909-100ML-F	
18B7 mouse anti-GXM antibody conjugated to Alexafluor 488			A kind gift from Dr. Arturo Casadevall (Johns Hopkins University)
PBS with 1% Bovine Serum Albumin (BSA)			PBS is the same recipe listed above (line 4) with 1% BSA added and filter sterilized.
Bovine Serum Albumin	Sigma-Aldrich	A9418	
Superfrost microscope slides	Fisher Scientific	12-550-143	
Glass coverslips	Corning	2855-18	#1.5 thickness
Clear nail polish or other non-toxic sealant			
Image Acquisition			
Immersion oil	Cargille	16484	
Light microscope with immersion oil objective	Zeiss		Zeiss Axio A1 with a Plan - NEOFLUAR 100x oil immersion NA 1.30 objective

Light microscope camera	Zeiss		Zeiss Axiocam ErCD camera
Confocal microscope with oil immersion objective	Zeiss		LSM 700 laser scanning confocal equipped with a Plan-Apochromat 63X NA 1.4 oil immersion DIC M27 objective.
Confocal microscope software	Zen 2009		
Confocal microscope camera	Nikon		Nikon Ti-Eclipse with a Intensilight epifluorescence illuminator (Nikon), CoolSNAP MYO microscope camera (Photometrics), Plan Apo 60x NA 1.40 oil immersion objective (Nikon) and 1.5x magnification changer.
Widefield imaging software	Nikon Elements (Nikon)		
Capsule Measurement			
Image editing software	Photoshop (Adobe)		
Microscope software for manual measurement	Axiovision (Carl Zeiss)		
Image analysis software for automated measurement	Aivia (DRVision Technologies)		
Spreadsheet software	Excel (Microsoft)		