

Materials List for:

Mass Isolation and *In Vitro* Cultivation of Intramolluscan Stages of the Human Blood Fluke *Schistosoma Mansoni*

Nathalie Dinguirard¹, Codie Heinemann¹, Timothy P. Yoshino¹

¹Department of Pathobiological Sciences, University of Wisconsin, School of Veterinary Medicine

Correspondence to: Timothy P. Yoshino at yoshinot@vetmed.wisc.edu

URL: https://www.jove.com/video/56345

DOI: doi:10.3791/56345

Materials

Name	Company	Catalog Number	Comments
Chernin's balanced salt solution (CBSS+)			For 1L of solution
2.8 g sodium chloride	Fisher Scientific	S271-3	Dissolve salts, except calcium chloride, and
0.15 g of potassium chloride	Sigma-Aldrich	P5405	sugars in 800 mL ddH2O
0.07 g sodium phosphate, dibasic anhydrous	Fisher Scientific	S374-500	Dissolve calcium chloride separately in 200
0.45 g magnesium sulfate heptahydrate	Sigma-Aldrich	M1880	mL ddH2O
0.53 g calcium chloride dihydrate	Mallinckrodt	4160	Slowly add calcium soln to the salt/ sugar soln with
0.05 g sodium bicarbonate	Fisher Scientific	S233-3	with constant mixing
1 g glucose	MP Biomedicals	152527	Adjust to pH 7.2 and filter sterilize using a
1 g trehalose	Sigma-Aldrich	T0167	0.22 μm disposable bottle-top filter
10 mL 100X penicillin/streptomycin	Hyclone	SV30010	Add filtered penicillin and streptomycin soln prior use
Incomplete Bge medium (Ibge)			For 900 mL solution
220 mL Schneider's Drosophila medium modified	Lonza	04-351Q	Mix Schneider's medium with 680 mL ddH2O
4.5 g lactalbumin enzymatic hydrolysate	Sigma-Aldrich	L9010	Add lactalbumin hydrolysate and galactose
1.3 g galactose	Sigma-Aldrich	G0625	Adjust to pH 7.2 and filter sterilize using a 0.22 µm pre-sterilized disposable bottle-top filter
Complete Bge medium (cBge)			For 100 mL of solution
90 mL Incomplete Bge medium			To heat-inactivate FBS: Incubate thawed FBS in
9 mL heat-inact. fetal bovine serum (FBS) (Optima)	Atlanta Biologicals	S12450	waterbath at 60°C for 1 hr while gently
1 mL 100X penicillin/streptomycin	Hyclone	SV30010	swirling the bottle every 10 min Aliquot heat-inactivated FBS into 15-mL tubes and store at -20°C. Mix medium + FBS and filter sterilize using a 0.22 µm pre- sterilized disposable bottle-top filter Add penicillin and streptomycin prior to use



Pond water (stock solution)			1L of stock solution
12.5 g calcium carbonate	Fisher Scientific	C64-500	Mix all salts in 1L of ddH2O
1.25 g magnesium carbonate	Fisher Scientific	M27-500	Note that the salts will not have completely
1.25 g sodium chloride	Fisher Scientific	S271-3	dissolved. Shake vigorously to suspend
0.25 g potassium chloride	Sigma-Aldrich	P5405	salts prior to making the working soln
Pond water (working solution)			1.5L of solution
0.8 mL stock solution pond water (shake prior use) in			Mix stock to ddH2O
1500 mL of ddH2O			Sterilize pond water by autoclaving (slow cycle)
1.5 mL of 100X penicillin/ streptomycin	Hyclone	SV30010	Add penicillin and streptomycin prior use
Saline solution (1.2% NaCl)			1.5L of solution
18 g sodium chloride in 1500 mL of ddH2O	Fisher Scientific	S271-3	Autoclave saline solution to sterilize
1.5 mL of 100X penicillin/ streptomycin	Hyclone	SV30010	Add penicillin and streptomycin prior use
Additional equipment and material:			
7-L mouse euthanizing chamber			Following approved IACUC protocol no. V001551
Mice	Taconic Biosciences		Swiss-Webster, female, 6-wk old, murine
CO ₂ tank and regulator			pathogen-free
24-well tissue culture plate	TPP	92424	
1-L volumetric flasks			
Light source (150W)	Chiu Tech Corp	Model F0-150	
Centrifuge, refrigerated, swinging bucket	Eppendorf	Model 5810R	
Centrifuge bottles (250 mL)	Nalgene		
15-mL centrifuge tubes, sterile	Corning	430053	
Sterile disposable transfer pipets	Fisher Scientific	1371120	
0.22 μm pre-sterilized disposable bottle-top filter	EMD Millipore	SCGPS05RE	
Stainless steel blender	Waring Commercial	Model 51BL31	
Blender cup, 100 mL capacity	Waring Commercial		
Inverted compound microscope	Nikon Instruments	Eclipse TE300	