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Version 4

Prepare NGM plates with Amphotericin B V.4

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Research Cancer UK / Wellcome Gurdon Institute media kitchen¹

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Protocol status: Working

We use this protocol and it's working

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Abstract

Prepare NGM plates with amphotericin B

From "<https://www.thermofisher.com/order/catalog/product/15290026?SID=srch-srp-15290026>":

"Amphotericin B is the generic version of Fungizone. "Fungizone" is a trademark of E.R. Squibb & Sons, LLC. Amphotericin B prevents the growth of fungi by causing an increase in fungal plasma membrane permeability. Gibco Amphotericin B is used to prevent the contamination of cell cultures by yeast and multicellular fungi. Gibco Amphotericin B contains 250 μg of amphotericin B and 205 μg of sodium deoxycholate per mL of distilled water. The recommended working concentration ranges from 0.25 to 2.50 $\mu\text{g}/\text{mL}$."

Materials

MATERIALS



Petri Dish **P212121 Catalog #LI-PD01100**



Magnesium Sulfate Heptahydrate Certified AR for Analysis Fisher Chemical **Fisher Scientific Catalog #M/1050/53**



Potassium Dihydrogen Orthophosphate Certified AR for Analysis Fisher Chemical **Fisher Scientific Catalog # P/4800/53**



NGM medium



Calcium chloride dihydrate for analysis EMSURE® ACSReag. Ph Eur **Merck Millipore (EMD Millipore) Catalog #102382**



Cholesterol powder BioReagent suitable for cell culture $\geq 99\%$ **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C3045**



Amphotericin B **Thermo Fisher Scientific Catalog #15290026**

Troubleshooting

Safety warnings



Make sure you know how to use the autoclave before starting this protocol.



1



NGM+Fungizone_plates(1L).xls

2

Ingredients			Quantity
NGM Media			1L
Cholesterol 5mg/ml		1ml	
1M CaCl ₂			1ml
1M MgSO ₄			1ml
1M KH ₂ PO ₄			25ml
Petri dish	30mm		as required
	50mm		as required
	90mm		as required
Fungizone	400 microlitres		1 microtube

3

First thing in the morning, melt 1L NGM in autoclave
Once melted, place in water bath set at 58°C, leave until cooled (-at least 2 hours) normally left until the afternoon.
Set out the plates on bench before starting pouring process.



	Put melted NGM media on stirrer
	Using 1ml gilson add the Cholesterol,replace the lid and return to the bench to give a good stir to dispense all the cholesterol.
	Add the KH ₂ PO ₄ using sterile 50ml syringe and 0.22µm filter.
	Stir for a few seconds until well mixed. Add Fungizone ensuring all is added to the NGM.
	Dispense media using a peristaltic pump. Add 4.20ml in 30mm plates, 9.00ml in 50mm plates, 4.50ml in 6 well plates and 2.5ml in 12 well plates. When set stack upside down in plastic trays.