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Prepare NGM plates with Amphotericin B V.4

Research Cancer UK / Wellcome Gurdon Institute media kitchen¹¹Wellcome Trust / Cancer Research UK Gurdon Institute
1 Works for me [dx.doi.org/10.17504/protocols.io.5zcg72w](https://doi.org/10.17504/protocols.io.5zcg72w)

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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 µg/mL."

MATERIALS

NAME	CATALOG #	VENDOR
Petri Dish	LI-PD01100	P212121
Magnesium Sulfate Heptahydrate Certified AR for Analysis Fisher Chemical	M/1050/53	Fisher Scientific
Potassium Dihydrogen Orthophosphate Certified AR for Analysis Fisher Chemical	P/4800/53	Fisher Scientific
NGM medium	View	
Calcium chloride dihydrate for analysis EMSURE® ACSReag. Ph Eur	102382	Merck Millipore
Cholesterol powder BioReagent suitable for cell culture ≥99%	C3045	Sigma Aldrich
Amphotericin B	15290026	Thermo Fisher Scientific

SAFETY WARNINGS

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

1 NGM+Fungizone_plates(1L).xls

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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 microtubbe

First thing in the morning, melt 1L NGM in autoclave
Once melted, place in water bath set at 58oC, leave until cooled (-at least 2hours) 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.



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