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Prepare NGM plates with fungizone V.3

Research Cancer UK / Wellcome Gurdon Institute media kitchen[1]

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1 Works for me dx.doi.org/10.17504/protocols.io.5sig6ce

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ABSTRACT

Prepare NGM plates with fungizon

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	
fungizone	View	
Calcium chloride dihydrate for analysis EMSURE® ACSReag. Ph Eur	102382	Merck Millipore
Cholesterol powder BioReagent suitable for cell culture ≥99%	C3045	Sigma Aldrich

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 CaCl2			1ml
1M MgSO4			1ml
1M KH2PO4			25ml
Petri dish	30mm		as required
	50mm		as required
	90mm		as required
Fungizone	400 microlitres		1 microtubbe

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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 KH2PO4 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.