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Prepare NGM plates with fungizone V.3

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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 fungizon





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

 fungizone

 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**

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.