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Prepare NGM plates with fungzizone V.3

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

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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 ≥99% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**C3045

Troubleshooting

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.