

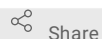


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# Making no peptone NGM for imaging plates

Bonnie Evans<sup>1</sup><sup>1</sup>Imperial College London

1 Works for me



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Behavioural Genomics

Bonnie Evans

## ABSTRACT

*C. elegans* is maintained in the laboratory on Nematode Growth Medium (NGM) agar seeded with bacteria. For imaging plates, NGM without bactopectone is used to prevent the seeded bacteria growing too much, resulting in poor imaging.

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## GUIDELINES

The quality of the imaging plates are crucial in producing good images that can later be analysed. It is very important to make sure there aren't any air bubbles trapped on the plates.

A	B	C
<b>Pre-autoclave</b>	For 1L:	For 500mL:
Sodium chloride	3g	1.5g
BioAgar	17g	8.5g
Sterile water	975mL	487.5mL

A	B	C
<b>Post-autoclave</b>	For 1L:	For 500mL:
5 mg/mL cholesterol (store at 4°C and away from light)	1mL	500uL
1M CaCl <sub>2</sub>	1mL	500uL
1M MgSO <sub>4</sub>	1mL	500uL
1M KPO <sub>4</sub>	25mL	12.5mL

## Pre-autoclave

- 1 Book the autoclave via the notebook on top of the machine. It will take around 2 hours for a 500ml bottle and 2.5 hours for 1L or larger bottles.
- 2 Take clean bottles and measuring cylinders from the glass kitchen - you will also need one bottle for the autoclave probe, which should be the size of the largest bottle you are autoclaving. Get sterile water from the media room.
- 3 Measure out all the pre-autoclave reagents and add to the bottle. Use a new weighing boat and spatula for each reagent. Add water last and mix thoroughly.
- 4 Add equivalent amount of water to the empty probe bottle.
- 5 Make sure the lid of your media bottle is not screwed completely when placing it inside the autoclave machine. Label with autoclave tape ('NGM agar Rm 5020'), and place over the lid to prevent it coming off in the autoclave.

## Autoclave

- 6 Turn ON the autoclave and touch the screen
- 7 If 'Fill with water' is on the screen, fill up the autoclave with sterile water until it reaches the grill/'Water Level OK' appears on the screen.

- 8 Place the probe bottle inside the autoclave with the temperature probe inside.
- 9 Place the bottles in the autoclave and make sure that the cap is not screwed completely.
- 10 Check the waste flask is not too full
- 11 Close the door
- 12 (On the screen) LOCK --> START --> MEDIA 121X15 BALLAST --> Start

#### Post-autoclave

- 13 When autoclave is finished, UNLOCK the door
- 14 Remove the probe and media bottles using gloves (the bottles will be hot)
- 15 Leave the agar on a lab bench to cool to around 55°C, ie the bottle is cool enough to hold for a second with a gloved hand
- 16 Add the post autoclave reagents
- 17 Try not to shake the bottle too much while mixing to avoid air bubbles.
- 18 The agar needs to be warm to be poured without blocking the tubings, so try to pour as quickly as possible and if not poured immediately put the bottle on a waterbath set to 60C until being used.