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Version 1

Making low peptone NGM for imaging plates V.1

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Protocol status: Working

We use this protocol and it's working

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Materials

Reagents:

For 500ml

A) Pre-Autoclave

- Sodium Chloride- 1.5g
- Difco Agar- 10g
- Difco Bactopeptone- 0.065g
- Cholesterol (5mg/ml in EtOH)- 0.5ml
- Deionized water- 482.5ml

B) Post-Autoclave

- 1M CaCl₂- 0.5ml
- 1M MgSO₄- 0.5ml
- 1M K₂HPO₄ (pH 6.0)- 12.5ml

Troubleshooting



Pre-Autoclave:

- 1
 - Book the autoclave (notebook on top of the machine).
 - Take clean flasks from the glass kitchen.
 - Measure all the pre-autoclave reagents and add to the flask (Use a new weighing boat and spatula for each reagent. Also, the cholesterol is kept in the fridge.)
 - Once water is added mix thoroughly and label with autoclave tape ('Low peptone NGM Rm 5020').

Using the autoclave:

- 2
 - Turn ON the autoclave
 - Make sure that the autoclave's probe bottle is the same size as the largest bottle you use and fill it with water.
 - Place the temperature probe in it.
 - Fill up the autoclave with water until it reaches the grill.
 - Place the bottles in the autoclave and make sure that the cap is not screwed completely.
 - Check the waste flask is not too full
 - Use 'media' program.
 - Press START.
 - It will take about 2 hours for 500ml to autoclave

Post autoclave:

- 3
 - When autoclave is complete, remove the probe flask
 - Make sure to wear gloves as the flask will be hot
 - Let the agar to cool to around 55°C and add the post autoclave reagents
 - Mix it well and start pouring onto small imaging plates (3.5ml in each) using a 3mm tube (See Protocol for plate pouring)