Making low peptone NGM for imaging plates

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Behavioural Genomics

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PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS TEXT

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 $Cacl_2$- 0.5ml
- 1M $MgSO_4$- 0.5ml
- 1M K_2HPO_4 (pH 6.0)- 12.5ml

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)