1 Liter CM^+

DasSarma Laboratory Protocol

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NOTE: 1 Liter CM^+ makes about 44 Petri Plates or 6 Large Square Plates

Materials:

250 g Sodium Chloride (NaCl)
20 g Magnesium Sulfate ($MgSO_4.7H_2O$)
3 g (Tri)Sodium Citrate ($Na_3C_6H_5O_7.2H_2O$)
2 g Potassium Chloride (KCl)
10 g Oxoid Neutralized Bacteriological Peptone (best weighed on weighing paper to prevent sticking)
Sodium Hydroxide solution (NaOH) for adjusting pH
Deionized water to 1 Liter
0.1 ml trace metal stock solution*

Methods:

1. Add NaCl into 1 Liter beaker with stir bar and place on stir plate.
2. Add 800 ml deionized water and stir until the salt is completely dissolved.
3. Add the $MgSO_4.7H_2O$, Trisodium citrate.$2H_2O$, KCl and Oxoid bacteriological peptone and stir until completely dissolved. (The trace metals will be added after autoclaving.)
4. Adjust pH to 7.2 using NaOH.
5. Bring volume up to 1 Liter.
6. Pour 500 ml into each of 2 1 Liter bottles so as to prevent overflow during autoclaving – this is due to the fact that the high salt content can cause superboiling.
7. Place caps loosely onto the tops of the bottles.
8. Autoclave at 120 lb/in^2 121°C for 20 minutes.
9. When the bottle contents cool to 65°C, add 0.05 ml trace metals into each 500 ml of media in each bottle.

To make plates:

Follow steps 1-5.
Label two Earlenmeyer flasks and add 1 stir bar and 10 g agar per flask (so a total of 20 g Agar/Liter).
Add 500 ml media to each flask, cover with aluminum foil and stir briefly – but not vigorously (as this will cause agar to stick to sides of flask) before autoclaving.
Follow steps 8, 9 above.

200 ml Trace Metal Solution

Note: the trace metals is what makes adds the "+" to CM. You can use the media without adding trace metals, but in the research laboratory we always add them.

1.32 g $ZnSO_4.7H_2O$
0.34 g $MnSO_4.H_2O$
0.78 g $Fe(NH_4)_2SO_4.6H_2O$
0.14 g $CuSO_4.5H_2O$

Stir metals in 1 N HCl and filter sterilize into an opaque bottle – or cover a clear bottle with aluminum foil.
You can store bottle on bench.
Be sure to shake before use as metals tend to settle in bottle.
Once prepared, the bottle can last you for years.