AIM: TO PREPARE LB AGAR MEDIA PLATES WITH THE ANTIBIOTIC AMPICILLIN

AMOUNT TO BE PREPARED: 250ml of LB Agar media and pour into 8x 9cm diameter petri-dishes

APPARATUS NEEDED: Weighing balance, Distilled water, Cotton, Conical flask, Magnetic stirrer, Magnetic bead, Magnetic bead retriever, Beaker, Measuring cylinder, Cotton, Aluminum foil, Autoclave, Laminar air flow, Micropipettes.

MATERIALS OR CHEMICALS REQUIRED AND THEIR USE:

1) Sodium Chloride (NaCl) (1.0%): Maintains the Osmolarity
2) Yeast Extract (0.5%): Source of minerals and vitamins
3) Tryptone (1.0%): Source of nitrogen and proteins
4) Agar (2%): Used as a solidifying agent.
5) Antibiotic: Ampicillin (final concentration in media:100µg/ml): Used for selecting bacteria containing plasmid DNA

PROCEDURE:

1. Calculate the amounts to be weighed for 250 ml of solution
2. Show the calculations to the teaching assistants to verify
3. Proceed as follows
4. Weigh appropriate amount of NaCl, yeast extract, tryptone into a conical flask. Use a separate weighing paper for each chemical
5. Before weighing out every chemical, rinse the spatula with distilled water and wipe it dry with tissue paper.
6. Dissolve the contents in approximately 200ml of distilled water (distilled water from dispensers) using magnetic stirrer & bead and then make up the volume to 250ml in measuring cylinder, transfer to conical flask.
7. After transferring the 250ml of dissolved media to the flask, add 5 gm Agar.
8. Cover the mouth of conical flask using cotton plug then wrap it with aluminum foil and label it accordingly.
9. Autoclave it at 121°C at 15 Lbs pressure for 15 minutes.
10. Then allow the solution to cool down (approximately up to 50°C) and add appropriate amount of the antibiotic ampicillin in to the media.
11. Label the underside of the plates LB-amp with a marker pen
12. Pour the ampicillin-containing LB agar media into 8 petri-dishes of 9cm diameter under aseptic conditions (laminar airflow).

Ampicillin stock solutions (100 mg / ml will be provided).
Calculate the amount to be added per 250 ml (Final concentration = 100µg/ml

AIM: TO PREPARE SOLUTIONS FOR PLASMID ISOLATION
(Solution-1, Solution-2, Solution-3 and TE buffer)

APPARATUS NEEDED: Weighing balance, Distilled water, Magnetic stirrer, Magnetic bead, Magnetic bead retriever, Beaker, Measuring cylinder, Micropipette, pH meter

Composition of the Solutions: (10 ml each to be prepared). Label the tubes.

1) SOLUTION 1:
   a) Glucose (50mM)
   b) Tris-HCl (25mM, pH 8.0)
   c) EDTA: (10mM, pH 8.0)

   Make up the volume to 10 ml using Sterile distilled water in the graduated tubes

2) SOLUTION 2:
   a) SDS (1 %)
   b) NaOH: (0.2N)

   Make up the volume to 10 ml using Sterile distilled water in the graduated tubes

3) SOLUTION 3:
   a) Potassium acetate (CH_3COOK) (3M)
   b) Glacial Acetic acid: 1.15ml (to obtain pH 5.5)

   Make up the volume to 10 ml using Sterile distilled water in the graduated tubes

4)TE Buffer (10ml) :
   10 mM Tris-Hcl (pH-8.0)
   1 mM EDTA (pH-8.0)

   Make up the volume to 10 ml using Sterile distilled water in the graduated tubes

The above solutions are to be made from stock solutions provided.

PROCEDURE:

-Calculate the amount of each solution to be added
-Show the calculation to the teaching assistants to verify
-prepare accordingly.

STOCK SOLUTIONS (4 sets of stock solutions will be provided by the lab):
Glucose (1M)
Tris-HCl (pH-8.0) (1M)
EDTA (pH-8.0) (0.5M)
NaOH (1N, 20ml)
CH3COOK (5M)
SDS (10%)
Glacial Acetic Acid

Each group of students will prepare solution 1, 2, 3 and TE buffer in 10ml tubes