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Biomedical Engineering

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Preparative steps: culture media, agar plates, antibiotics and glycerol

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Preparative steps

1 Preparation of Culture Media

1.1 Luria-Bertani (LB) medium

For 1L
- 10 g peptone
- 10 g NaCl
- 5 g yeast extract
- Add 1L H_2O

1.2 2YT medium (for protein expression)

For 1L
- 16 g peptone
- 5 g NaCl
- 10 g yeast extract
- Add 1L H_2O

2 Preparation of Agar Plates

2.1 LB-agar

For 1L
- 10 g peptone
- 10 g NaCl
- 5 g yeast extract
- 15 g bacto-agar
- Add 1L H_2O

Both media and agar need to be autoclaved (sterilization).

2.2 Pouring agar plates

- After autoclaving the LB-agar at 121 °C for 20 minutes, let the agar cool down to ~50°C. Make sure the agar does not start solidifying
- Add kanamycin and /or chloramphenicol to the liquid LB-agar and slowly mix.
- Pour the LB-agar in the petri dishes until the bottom is well covered. Work near the Bunsen burner flame
- Close the lid after filling the plate. Let the agar solidify for ~1 hour on the bench
- Transfer the plates to a bag, in which they should be placed upside down
- Store the plates in the fridge (4°C)

3 Preparation Antibiotics

3.1 Kanamycin

30 mg/mL stock solution in H_2O. Final concentration in cultures and LB-agar: 30 µg/mL
Filter stock solution using 0.2 µm filter.

3.2 Chloramphenicol (for protein expression)

25 mg/mL stock solution in ethanol. Final concentration in cultures and LB-agar: 25 µg/mL
Filter stock solution using 0.2 µm filter.

4 Preparation of Glycerol Stocks

- Prepare a 50% v/v glycerol solution in dH_2O and mix well
- Take 500 µL of desired bacterial broth to form stock.
- Add 500 µL of 50% glycerol stock to the broth and mix well
- Snap-freeze using liquid nitrogen and store at -80°C