Microbiological Media

Recipes for commonly used bacterial media

Prepare liquid media according to following recipes.
The given amounts of the ingredients are for a final volume of 1 L.

LB Medium (Luria-Bertani Medium)

To 950 mL deionized water add:

10 g	Peptone from casein	Cat.no. 48600
5 g	Yeast Extract	Cat.no. 24540
10 g	NaCl	Cat.no. 30183

- Dissolve the reagents by stirring on a magnetic stirrer.
- Adjust the pH to 7.0 with approximately 0.2 mL of 5 N NaOH.
- Fill up to a final volume of 1 L with deionized water.
- Sterilize by autoclaving.

2 x YT Medium

To 900 mL deionized water add:

6 g	Peptone from casein	Cat.no. 48600
10 g	Yeast Extract	Cat.no. 24540
5 g	NaCl	Cat.no. 30183

- Dissolve the reagents by stirring on a magnetic stirrer.
- Adjust the pH to 7.0 with approximately 0.2 mL of 5 N NaOH.
- Fill up to a final volume of 1 L with deionized water.
- Sterilize by autoclaving.

SOB Medium

(for the production of transformation competent bacteria, Ref. 1)

To 950 mL deionized water add:

20 g	Peptone from casein	Cat.no. 48600
5 g	Yeast Extract	Cat.no. 24540
0.5 g	NaCl	Cat.no. 30183

- Dissolve the reagents by stirring on a magnetic stirrer.
- Adjust the pH to 7.0 with approximately 0.2 mL of 5 N NaOH.
- Fill up to a final volume of 1 L with deionized water.
- Sterilize by autoclaving.
- Just before use add:

2.5 mM KCl	2.5 mL from a 1 M stock solution
10 mM $MgCl_2$	10 mL from a 1 M stock solution
10 mM $MgSO_4$	10 mL from a 1 M stock solution

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Recipe for Preparation of Agar plates

1. Agar plates are prepared by adding 15 g of Agar-Agar per 1L of liquid medium.
2. Sterilize the mixture by autoclaving.
3. Dissolve melted agar by gentle shaking to avoid air bubbles.
4. Add thermolabile substances such as antibiotics (for example ampicillin to a final concentration of 50 mg/mL) after cooling down the medium to 45 – 50 °C.
5. Pour 25 to 35 mL of the medium per 90-mm-plate.
6. To remove bubbles on the surface flame the medium before hardening with a Bunsen burner.
7. The hardened plates are stored at 4 °C in an inverted position; the stability depends on the antibiotic used.
8. 1 to 2 hours before inoculating with bacteria the plates should be placed at room temperature.
9. Remove liquid by wiping off condensation from the lids of the plates.
10. Inoculate with 0.1 mL bacteria suspension and wait for 30 minutes until the liquid has disappeared.
11. Invert the plates and incubate them for 12 to 16 hours at 37 °C.

Recipe for Preparation of Topagar

1. Topagar is prepared with 7 g Agar-Agar per 1 L of liquid medium, according to the recipes given for the preparation of agar plates.
2. In case of a 90-mm-plate 3 mL of topagar are used.
3. The sterilized hot topagar is incubated in a water bath at 47 °C before use.
4. When the temperature is reached add plating bacteria and phages or solutions desired.
5. Pour carefully onto a pre-made agar plate to ensure the overall distribution of the topagar.
6. Allow the topagar to harden for 5 minutes at room temperature.
7. Invert the plate and incubate at 37 °C for 12 to 16 hours.

Recipe for Preparation of Buffers

1 M $MgCl_2$ (Cat.no. 39771)
- Dissolve 203.3 g of $MgCl_2$ in 800 mL of H_2O
- Adjust the volume to 1 L with H_2O and sterilize by autoclaving

1 M $MgSO_4$ (Cat.no. 39773)
- Dissolve 120.4 g of $MgSO_4$ in 800 mL of H_2O
- Adjust the volume to 1 L with H_2O and sterilize by autoclaving

1 M KCl (Cat.no. 26868)
- Dissolve 74.6 g of KCl in 800 mL of H_2O
- Adjust the volume to 1 L with H_2O and sterilize by autoclaving

Reference:
1) D. Hanahan (1985) Techniques for transformation of E.coli. DNA cloning, Col I. A practical approach. Glover, D.M. (ed.), IRL Press, Washington D.C., 109 - 125

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