

Instructions for Use

REF - BM5280

LB Agar (Miller)

Bottled Media

1. Intended Use

BM5280 LB Agar (Miller) is used for the cultivation and maintenance of *Escherichia coli* for molecular microbiology.

2. Composition*

Ingredient	g/L
Tryptone	10.0
Yeast extract	5.0
Sodium chloride	10.0
Agar	15.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

BM5280 is based on the formulation developed by Miller ⁽¹⁾ and designed to meet the requirements of nutritionally fastidious *E. coli* strains typically used in molecular manipulation experiments. These strains of *E. coli* are generally derived from stain K12 and have an absolute requirement for exogenous vitamin B. Higher concentrations of sodium chloride in Miller's medium allows for selection of the salt level and osmolality which best suit the strain under investigation.

4. Principle

Enzymatic digest of casein and yeast extract supply the required nitrogen, carbon, vitamin B, and other essential growth factors required for the growth on nutritionally fastidious *E. coli* strains. Higher concentrations of sodium chloride in Miller's medium allows for selection of the salt level and osmolality which best suit the strain under investigation.

5. Physical Characteristics

	Appearance and Colour	pH
Medium	Straw firm gel	7.0 ± 0.2

6. Materials Provided

BM5280 LB Agar (Miller) is available in packs of 6 x 300ml in 500ml Sirop Bottles (product code: BM5280-J500-300). Each bottle is ink-jet printed or labelled with (abbreviated) product name, product code, lot number and expiry date.

7. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., sterile loops or swabs, collection containers, incubators, and quality control organisms.

8. Specimens

BM5280 LB Agar (Miller) is suitable for the testing of the following specimens:

- Isolated colonies or stock cultures.

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

9. Test Procedures and Interpretation of results

Heat the agar in a boiling water bath or similar until the agar has fully melted. Mix, and allow to cool to approximately 45°C. If selective agents or additional nutrient components are required, aseptically add sterile components to the cooled agar and mix gently but thoroughly. Pour to petri dishes or slopes and allow to set. Use plates or slopes as directed by the protocol being followed.

10. Quality Control

Organism	Incubation	Result (Specificity)
<i>E. coli</i> (NCTC 12241)	37 ± 1°C for 18-24 hours	Growth: Cream colonies
<i>S. aureus</i> (NCTC 12981)	37 ± 1°C for 18-24 hours	Growth: White colonies

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

11. Performance

To fully verify the performance of BM5280 LB Agar (Miller), samples were tested to assess colony morphology and recovery when incubated at $37 \pm 1^\circ\text{C}$ for 18-24 hours. All samples showed good recovery and the correct morphology of the required test organisms: *Escherichia coli* (NCTC 12241) and *Staphylococcus aureus* (NCTC 12981). Therefore, it can be concluded that BM5280 LB Agar (Miller), meets performance criteria when used according to the instructions outlined above.

12. Limitations of the Medium

- BM5280 is a non-selective, nutritious medium so other organisms including bacteria and fungi may grow on the agar. Use full aseptic technique when adding sterile supplements, during dispensing, and when handling/inoculating the plate.
- Due to natural variation, some strains may grow poorly on this medium.

13. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSHH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

Refer to E&O BM5280 Bottled Media Material Safety Data Sheet.

14. Storage conditions and Shelf life

Store BM5280 in the original bottle with the lid tightly closed at between 2 and 8°C . Kept under these conditions it may be used up to date of expiry shown on product label.

Dispose of in accordance with local and national authority requirements. Do not use medium if it is contaminated, discoloured or out of date. Additionally, do not use medium if it has been stored inappropriately or the packaging has been damaged.

15. References

1. Miller, J.H. (1972). Experiments in Molecular Genetics. Cold Spring Harbour Laboratory. New York: Cold Spring Harbour.

Version History*

001 06/08/24 - New Document Created

*Note: minor typographical, grammatical, and formatting changes are not included in the revision history.



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IFU/BM5280 REV. 001

TABLE OF APPLICABLE SYMBOLS

 REF Catalogue number	 LOT Batch code		 Manufacturer	 Use by
 Temperature limitation	 Contents sufficient for <n> tests	 Consult Instructions for Use	 Keep away from direct light	 Store in a dry place