EOSIN METHYLENE BLUE AGAR

EMBA-00I-500

- **Principle**

Eosin Methylene Blue Agar is a selective and differential medium used for the isolation and differentiation of Gram-negative enteric bacteria, particularly coliforms, in clinical, food, water and environmental samples. The formulation contains peptone as a source of carbon, nitrogen, vitamins and essential minerals required for bacterial growth. Lactose and sucrose are included as fermentable carbohydrates, allowing the differentiation of organisms based on their ability to ferment these sugars. Dipotassium phosphate acts as a buffering agent to maintain pH stability during microbial metabolism.

Eosin Y and methylene blue serve both as selective agents and as pH indicator dyes. These compounds partially inhibit the growth of Gram-positive microorganisms and react to changes in pH resulting from carbohydrate fermentation. The medium differentiates lactose fermenters from non-lactose fermenters through visible colour changes associated with acid production. Some Gram-negative bacteria are unable to ferment lactose or do so slowly; therefore, sucrose is incorporated as an additional fermentable carbohydrate to improve differentiation.

Organisms capable of fermenting lactose and/or sucrose produce acid, lowering the pH in the vicinity of the colonies. Under acidic conditions, eosin and methylene blue form a precipitated dye complex, resulting in dark purple to black colonies. Escherichia coli typically produces colonies with a characteristic green metallic sheen due to strong acid production and dye precipitation. In contrast, lactose non-fermenting organisms, such as Salmonella species, do not produce significant acid. Instead, deamination of proteins may raise the local pH, leading to dissolution of the dye complex and the formation of colourless or translucent colonies. Agar serves as the solidifying agent.

- **Regulatory compliance**

This product is manufactured under a quality management system in accordance with ISO 9001 and ISO 13485, and its formulation and quality control comply with applicable international standards, such as ISO 11133, where relevant.

- **Composition**

Ingredients	**g/L**
Peptone	10.00
Lactose	5.00
Sucrose	5.00
Dipotassium Phosphate	2.00
Eosin Y	0.40
Methylene Blue	0.065
Agar	13.50

- **Preparation**

Dissolve 36 grams in 1,000 ml distilled water check. Boil to dissolve the medium completely and sterilize by autoclaving at 15 lbs pressure (121 °C) for 15 min, cool it to 42-45 °C and distribute aseptically. Ensure complete solidification and inoculate test sample aseptically.

- **Applications and use**

Recommended for the isolation, enumeration and differentiation of members of Enterobacteriaceae from clinical and non-clinical samples.

- **Quality control**

Solubility	w/o rests
Appearance	Fine powder
Colour of the dehydrated medium	Pinkish purple
Colour of the prepared medium	Purple-greenish
Final pH (25 °C)	7.2 ± 0.2

- **Microbiological test**

Cultural characteristics observed after incubation at 33-37°C for 18-24 hours. Inoculum 50-100 CFU.

Microorganism	ATCC	Growth	Recovery	Colony Colour
Escherichia coli	8739	Luxuriant	≥ 70%	Black colour with Shiny Metallic Green Sheen
Escherichia coli	25922	Luxuriant	≥ 70%	Black colour with Shiny Metallic Green Sheen
Salmonella typhimurium	14.028	Good	≥ 50%	Translucent Amber Coloured Colonies
Klebsiella aerogenes	13.048	Good	≥ 50%	Pinkish without sheen
Staphylococcus aureus	25.923	Inhibited	-	-

- **Storage**

The product is highly hygroscopic; keep the container always closed and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Temp. Min.:2 °C Temp. Max.:25 °C.

Note: Sterilize media immediately after reconstitution.

- **Bibliography**

Atlas, R. M. (2004). Handbook of microbiological media. CRC press.

Atlas, R. M. (2005). Handbook of media for environmental microbiology. CRC press.

Difco Manual (1998). 11th Edition. Difco Laboratories., Division of Becton Dickinson and Company, Sparks, Maryland, USA.

Rand, M. C., Arnold E. Greenberg, and Michael J. Taras. Standard methods for the examination of water and wastewater. Prepared and published jointly by American Public Health Association, American Water Works Association, and Water Pollution Control Federation., 1976.

- **Product use limitation**

This product is developed, designed and supplied exclusively for research use only. It is not intended for diagnostic applications or drug development, and it is not suitable for administration to humans or animals.