

Eosine Methylene Blue (EMB) Agar

A differential medium which distinguishes between gram-negative pathogenic microbes in a short period

REF: BS.1/EA01.100.0100	100 Gram	REF: BS.1/EA01.500.0500	500 Gram
REF: BS.1/EA01.250.0250	250 Gram		

CLINICAL SIGNIFICANCE

EMB Agar (Eosin Methylene Blue Agar) is recommended for the isolation and differentiation of gram-negative enteric bacilli from clinical and nonclinical specimens and slightly inhibiting the growth of gram-positive bacteria. EMB is used in testing the quality of water, especially in determining if the water is contaminated by pathogenic microorganisms. It is also useful in differentiating between lactose fermenting and non-lactose fermenting bacteria.

METHOD PRINCIPLE

Methylene blue and Eosin-Y inhibit gram-positive bacteria to a certain degree. These dyes act as differential indicators due to the fermentation of carbohydrates. The ratio of eosin and methylene blue is approximately 6:1. Sucrose is added to the medium as an alternative carbohydrate source for typically lactose-fermenting, gram-negative bacilli, which do not ferment lactose or do so slowly. The coliforms produce purplish black colonies due to the consumption of methylene blue-eosin dye complex, when the pH drops. The dye complex is absorbed into the colony. Non-fermenters probably raise the pH of surrounding medium by oxidative deamination of protein, which solubilizes the methylene blue-eosin complex causing the appearance of colorless colonies. Some strains of *Salmonella* and *Shigella* species do not grow in the presence of eosin and methylene blue. Further tests are required to confirm the isolates.

MEDIA COMPOSITION

Item	Formula per liter of medium
Peptone	10 gm
Dipotassium hydrogen phosphate	2 gm
Lactose	5 gm
Sucrose	5 gm
Eosin-Y	0.4 gm
Methylene blue	0.065 gm
Agar	13.5 gm

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment.

For further information, refer to the EMB Agar material safety data sheet.

STORAGE AND STABILITY

BioScien EMB Agar should be stored between 10-30°C in a firmly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to avoid lump development due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in a dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Final pH 7.2±0.2 at 25°C

PREPARATION

Suspend 35.96 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. AVOID OVERHEATING. Cool to 45-50°C and shake the medium to oxidize the methylene blue (i.e. to restore its blue color) and to suspend the flocculent precipitate. If the EMB Agar is going to be inoculated on the same day, it can be used without autoclaving.

NOTE: Store the medium away from light to avoid photo-oxidation

Deterioration

The color of **BioScien** EMB Agar is light pink to purple homogeneous free flowing powder. Prepared Media is reddish purple colored with greenish cast and finely dispersed precipitate forms in petri dishes. If there are any physical changes for powder or signs of deterioration (shrinking, cracking, or discoloration), and contaminations for hydrated media, discard the medium.

SPECIMEN

Faecal samples, food samples and water samples.

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile petri dishes
- Incubator
- Autoclave

PERFORMANCE CHARACTERISTICS

Microorganisms	Result	Colony color
<i>Klebsiella aerogenes</i> ATCC 13048	Good growth	Pink color, without sheen
<i>Klebsiella pneumoniae</i> ATCC 13883	Good growth	Pink color, mucoid
<i>Escherichia coli</i> ATCC 25922	Luxuriant growth	Purple color with black centre
<i>Proteus mirabilis</i> ATCC 25933	Luxuriant growth	Colorless
<i>Salmonella Typhimurium</i> (ATCC 14028)	Luxuriant growth	Colorless
<i>Staphylococcus aureus</i> (ATCC 6538)	Inhibited	-

QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control still out of range please contact **BioScien** technical support.

REFERENCES

1. Isenberg (Eds.), 1992, Clinical Microbiology Procedures Handbook, Vol. 1, American Society for Microbiology, Washington, D.C.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock, D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
3. American Public Health Association, American Water Works Association and Water Pollution Control Federation, (1975). 2017. Standard methods for the examination of water and wastewater, 23rd ed. American Public Health Association, Washington, D.C
4. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
5. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C

SYMBOLS IN PRODUCT LABELLING	
 For in-vitro diagnostic use	 Number of <n> test in the pack
 Batch Code/Lot number	 Caution
 Catalogue Number	 Do not use if package is damaged
 Temperature Limitation	 Consult Instruction for use
 Expiration Date	
 Manufactured by	