EMB Agar

Intended Use
Eosin Methylene Blue (EMB) Agar is a slightly selective and differential medium recommended for the isolation, cultivation and differentiation of Gram-negative enteric bacilli from clinical and non-clinical specimens.

Summary
Eosin Methylene Blue (EMB) agar was originally developed by Holt-Harris and Teague. Eosin Y and Methylene Blue are the two dyes incorporated in these media. This formulation gives a sharp and distinct differentiation between the colonies of lactose fermenting and non-lactose fermenting microorganisms.

Principle
The media contain Eosin Y and Methylene Blue dyes that inhibit Gram-positive bacteria to a limited degree. In addition, these dyes also serve as differential indicators in response to lactose/sucrose fermentation by the microorganisms. Sucrose is added to the media as an alternative carbohydrate source for typical lactose fermenting, Gram-negative bacilli, which on occasion may not ferment lactose or may do slowly. Lactose fermenters will drop the pH of the media which results in the formation of purplish black colonies due to absorption of methylene blue-eosin dye complex, while Lactose non-fermenters probably raise the pH of surrounding medium by oxidative deamination of protein, which solubilizes the methylene blue-eosin complex resulting in colourless colonies.

Formula*

Ingredients	**g/L**
Tryptone	10.0
Dipotassium Phosphate	2.0
Lactose	5.0
Sucrose	5.0
EosinY	0.4
Methylene Blue	0.065
Agar	13.5
Final pH (at 25°C)	7.2 ± 0.2

*Adjusted to suit performance parameters.

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of specimen
Water samples and Clinical samples.

Specimen Collection and Handling
Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions
1. Suspend 35.96 g of the powder in 1000 mL purified / distilled water.
2. Mix thoroughly until the suspension is uniform.
3. Heat with frequent agitation to dissolve the powder completely. AVOID OVER HEATING.
4. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
5. Cool to 50°C and shake the medium to oxidize the Methylene Blue and to suspend the flocculent precipitate.
6. Pour into sterile petridishes.

Quality Control

Dehydrated Appearance: Light pink to lilac coloured, homogeneous, free flowing powder.
Prepared Appearance: Deep red-brown, slightly opalescent gel with or without fine precipitate, forms in the petridishes.
Growth Promotion Test: Growth promotion is carried out in accordance with the harmonized method of USP/EP/JP/IP and growth is observed after incubation at 30°C-35°C for 18 to 24 hours.
Growth Promoting Properties: The test results observed are within the specified temperature and shortest period of time specified in the test, inoculating ≤100 cfu of appropriate microorganism at 30°C-35°C for 18 hours.
Indicative Properties: The test results observed are within the specified temperature and time, inoculating ≤100 cfu of appropriate microorganism.

Organism (ATCC)	**Growth**	**Colour of Colony**
Salmonella enterica subsp. *enterica* serovar *Typhimurium* (14028)	Good	Colourless
Salmonella enterica subsp. *enterica* serovar *Typhimurium* (23564)	Good	Colourless
Enterococcus faecalis (29212)	Partial Inhibition	Pinkish purple, pin point
Klebsiella pneumoniae subsp. *pneumoniae* (10031)	Good	Pink with blue centre, mucoid
Klebsiella aerogenes (13048)	Good	Pink with blue centre
Staphylococcus aureus subsp. *aureus* (25923)	Complete Inhibition	-
Escherichia coli (25922)	Good	Purple with black centre and green metallic sheen
Proteus mirabilis (25933)	Good	Colourless

Note: For good growth - Growth obtained on test media should not differ by a factor greater than 2 from calculated value for a standardized inoculum.

Interpretation of Results

1. Coliforms produce blue-black colonies due to the taking up of an Eosin methylene blue dye complex by the bacterial cells when the pH drops.
2. *Salmonella* and *Shigella* colonies appear colourless or have a transparent amber colour.
3. *Escherichia coli* colonies may show a characteristic green metallic sheen due to rapid fermentation of lactose.
4. Some Gram-positive bacteria, such as faecal *Streptococci, Staphylococci* and yeasts, usually form pin point colonies.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions/Limitations

1. Store the prepared medium away from light to avoid photo-oxidation.
2. If EMB Agar is inoculated on the same day, as it is prepared, it may be used without autoclave sterilization.
3. A number of non-pathogenic, lactose non-fermenting Gram-negative bacteria will grow on this medium and must be distinguished from the pathogenic bacterial strains by additional biochemical tests.
4. Serial inoculation may be required to assure adequate isolation of mixed flora samples.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201050060100	Dehydrated Culture Media	100 g
201050060500	Dehydrated Culture Media	500 g
201050062500	Dehydrated Culture Media	2.5 k