

# TM 314 – EMB BROTH

#### **INTENDED USE**

For isolation of gram-negative enteric bacteria from clinical and non-clinical specimens.

## PRODUCT SUMMARY AND EXPLANATION

Eosin Methylene Blue (EMB) media were originally devised by Holt-Harris and Teague and further modified by Levine. The above media are combination of the Levine and Holt-Harris and Teague formulae which contains peptic digest of animal tissue and phosphate as recommended by Levine and two carbohydrates as suggested by Holt-Harris and Teague.

EMB Broth has a similar composition as EMB Agar except agar. Methylene blue and Eosin-Y inhibit gram-positive bacteria to a limited degree. These dyes serve as differential indicators in response to the fermentation of carbohydrates. The ratio of eosin and methylene blue is adjusted approximately to 6:1. Sucrose is added to the medium as an alternative carbohydrate source for typically lactose-fermenting, gram-negative bacilli, which on occasion do not ferment lactose or do so slowly. The coliforms produce purplish black broth due to taking up of methylene blue-eosin dye complex, when the pH drops. Non-fermenters probably raise the pH of surrounding medium by oxidative deamination of protein, which solubilizes the methylene blue-eosin complex resulting in colourless broth. Some strains of *Salmonella* and *Shigella* species do not grow in the presence of eosin and methylene blue.

#### **COMPOSITION**

Ingredients	Gms / Ltr		
Peptone	10.000		
Lactose	5.000		
Sucrose	5.000		
Dipotassium hydrogen phosphate	2.000		
Eosin-Y	0.400		
Methylene blue	0.065		

# **PRINCIPLE**

The medium consists of Peptone which serves as source of carbon, nitrogen, and other essential growth nutrients. Lactose and sucrose are the sources of energy by being fermentable carbohydrates. Eosin-Y and methylene blue serve as differential indicators. Dipotassium hydrogen phosphate buffers the medium.

# **INSTRUCTION FOR USE**

- Dissolve 22.46 grams in 1000 ml purified / distilled water.
- Mix until suspension is uniform. Heat if necessary to dissolve the medium completely.
- Dispense in tubes or flasks as desired. Sterilize by autoclaving at 15 psi pressure (121° C) for 15 minutes. AVOID
  OVERHEATING.
- Cool to 45-50°C. Shake the medium in order to oxidize the methylene blue (i.e. to restore its blue colour) and to suspend the flocculant precipitate.
  - Precaution: Store the medium away from light to avoid photo-oxidation.

#### **QUALITY CONTROL SPECIFICATIONS**















**Appearance of Powder** : Light pink to purple homogeneous free flowing powder.

: Reddish purple coloured, opalescent solution with greenish cast and finely Appearance of prepared medium

dispersed precipitate in tubes.

pH (at 25°C) : 7.2 ± 0.2

#### **INTERPRETATION**

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Colour of medium	Incubation Temperature	Incubation Period
Escherichia coli	25922	50-100	Luxuriant	Purple with green metallic sheen	35-37°C	18-24 Hours
Klebsiella aerogenes	13048	50-100	Good	Pink	35-37°C	18-24 Hours
Klebsiella pneumoniae	13883	50-100	Good	Pink	35-37°C	18-24 Hours
Proteus mirabilis	25933	50-100	Luxuriant	Colourless	35-37°C	18-24 Hours
Salmonella Typhimurium	14028	50-100	Luxuriant	Colourless	35-37°C	18-24 Hours
Staphylococcus aureus subsp. aureus	25923	>=10 <sup>3</sup>	Inhibited	-	35-37°C	18-24 Hours

## **PACKAGING:**

In pack size of 100 gm and 500 gm bottles.

#### **STORAGE**

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

### **DISPOSAL**

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.







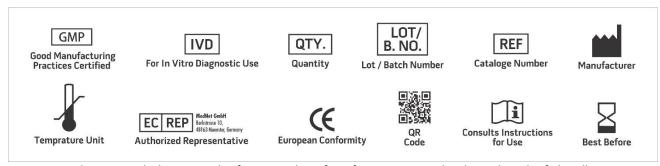






#### **REFERENCES**

- 1. Holt-Harris and Teague, 1916, J. Infect. Dis., 18: 596.
- 2. Howard B.J., 1994, Clinical and Pathogenic Microbiology, 2nd ed., Mosby Year Book, Inc.
- 3. Levine, 1918, J. Infect. Dis., 23:43.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices. \*For Lab Use Only

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