

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.
Appearance of prepared medium : Reddish purple coloured, opalescent solution with greenish cast and finely 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
<i>Escherichia coli</i>	25922	50-100	Luxuriant	Purple with green metallic sheen	35-37°C	18-24 Hours
<i>Klebsiella aerogenes</i>	13048	50-100	Good	Pink	35-37°C	18-24 Hours
<i>Klebsiella pneumoniae</i>	13883	50-100	Good	Pink	35-37°C	18-24 Hours
<i>Proteus mirabilis</i>	25933	50-100	Luxuriant	Colourless	35-37°C	18-24 Hours
<i>Salmonella</i> Typhimurium	14028	50-100	Luxuriant	Colourless	35-37°C	18-24 Hours
<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	25923	≥10 ³	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.

 GMP Good Manufacturing Practices Certified	 IVD For In Vitro Diagnostic Use	 QTY. Quantity	 LOT/ B. NO. Lot / Batch Number	 REF Catalogue Number	 Manufacturer
 Temperature Unit	 EC REP MedNet GmbH Buckstrasse 10, 48163 Münster, Germany Authorized Representative	 European Conformity	 QR Code	 Consults Instructions for Use	 Best Before

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**
Revision: 08 Nov., 2019