# TM 336 – EMB AGAR

## INTENDED USE

For differential isolation of gram-negative enteric bacteria from clinical and nonclinical samples.

## PRODUCT SUMMARY AND EXPLANATION

Eosin Methylene Blue (EMB) Agar was originally devised by Holt-Harris and Teague and further modified by Levine. The above medium is a combination of the Levine and Holt-Harris and Teague formulae which contains peptone and phosphate as recommended by Levine and two carbohydrates as suggested by Holt-Harris and Teague. 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 colonies due to taking up 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 resulting in colourless 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.

## COMPOSITION

| Ingredients | Gms / Ltr |
|---|---|
| **Peptone** | 10.000 |
| **Dipotassium hydrogen phosphate** | 2.000 |
| **Lactose** | 5.000 |
| **Saccharose (Sucrose)** | 5.000 |
| **Eosin - Y** | 0.400 |
| **Methylene blue** | 0.065 |
| **Agar** | 13.500 |

## 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. Phosphate buffers the medium.

## INSTRUCTION FOR USE

- Dissolve 35.96 grams in 1000 ml purified / distilled water.
- Mix until suspension is uniform. Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes. AVOID OVERHEATING.
- Cool to 45-50°C and shake the medium in order to oxidize the methylene blue (i.e. to restore its blue colour) and to suspend the flocculent precipitate. (If EMB Agar is inoculated on the same day, it may be used without autoclave sterilization).
  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 gel with greenish cast and finely dispersed precipitate forms in Petri plates.
**pH (at 25°C)** : 7.2 ± 0.2

## INTERPRETATION

Cultural characteristics observed after incubation.

| Microorganism | ATCC | Inoculum (CFU/ml) | Growth | Recovery | Colour of colony | Incubation Temperature | Incubation Period |
|---|---|---|---|---|---|---|---|
| *Klebsiella aerogenes* | 13048 | 50-100 | Good | 40-50% | Pink, without sheen | 35-37°C | 18-24 Hours |
| *Escherichia coli* | 25922 | 50-100 | Luxuriant | >=70% | Purple with black center and green metallic sheen | 35-37°C | 18-24 Hours |
| *Klebsiella pneumoniae* | 13883 | 50-100 | Good | 40-50% | Pink, mucoid | 35-37°C | 18-24 Hours |
| *Proteus mirabilis* | 25933 | 50-100 | Luxuriant | >=70% | Colourless | 35-37°C | 18-24 Hours |
| *Salmonella* Typhimurium | 14028 | 50-100 | Luxuriant | >=70% | Colourless | 35-37°C | 18-24 Hours |
| *Staphylococcus aureus subsp. aureus* | 25923 | >=$10^4$ | Inhibited | 0% | - | 35-37°C | 18-24 Hours |

## PACKAGING:

In pack size of 100 gm and 500 gm bottles.

## STORAGE

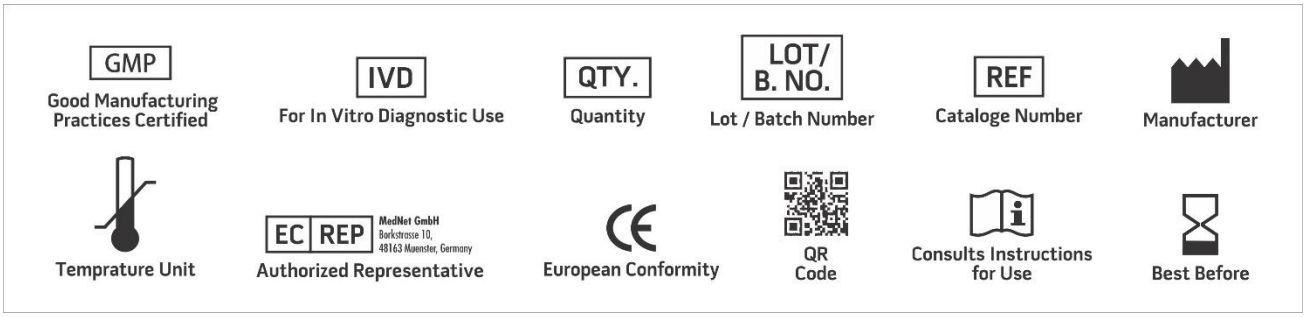
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided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