PRODUCT SPECIFICATION SHEET

Eosin Methylene Blue Agar (DM092)

Intended Use
Eosin Methylene Blue Agar (EMB Agar) (DM092) is a selective and differential medium used for the isolation, cultivation and differentiation of gram-negative enteric bacteria from clinical as well as non clinical samples.

Product Summary and Explanation
Eosin-Methylene blue (EMB) agar was first developed by Holt-Harris and Teague for the differentiation of enteric microorganisms.[1] They use lactose and sucrose with two indicator dyes, Eosin Y and Methylene Blue. Levine formulated this medium by removing the sucrose,[2] doubling the concentration of lactose and is used for the differentiation of *Escherichia coli* and *Enterobacter aerogenes*. 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. 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. 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. This medium is recommended for the detection, enumeration and differentiation of members of the coli form group by American Public Health Association.[3, 4, 5]

Principles of the Procedure
Peptic digest of animal tissue provides carbon, nitrogen, and other essential growth nutrients in the medium. Lactose and sucrose are the fermentable carbohydrates which are the sources of energy. Eosin-Y and methylene blue serve as differential indicators in response to the fermentation of lactose and/or sucrose by microorganisms. Phosphate buffers the medium. The ratio of eosin-Methylene blue is adjusted to approximately 6:1. These indicators also serve to inhibit the growth of most Gram positive organisms to a limited degree. 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. Nonfermenters probably raise the pH of surrounding medium by oxidative deamination of protein, which solubilizes the methylene blue-eosin complex resulting in colourless colonies.[3] *Escherichia coli* colonies may show a characteristic green metallic sheen due to the rapid fermentation of lactose. Some gram-positive bacteria, such as fecal streptococci, staphylococci and yeasts, will grow on this medium and usually form pinpoint colonies. A number of non-pathogenic, lactose-nonfermenting gram-negative bacteria will grow on this medium and must be distinguished from the pathogenic bacterial strains by additional biochemical tests.

Formula / Liter

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.00
Lactose	5.00
Sucrose	5.00
Dipotassium Phosphate	2.00
Eosin Y	0.40
Methylene Blue	0.065
Agar	13.50
Final pH: 7.2 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT, mainly irritating to eyes, respiratory system, and skin. Handle in accordance with good laboratory hygiene and safety practice. Wash hands before breaks and at the end of workday. To protect, use safety glasses and gloves during handling.
3. Use personal protective equipment. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Avoid breathing dust.
4. Do not let product enter drains. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.
5. Store the medium away from light to avoid photo-oxidation.

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Directions

1. Suspend 35.96 g of the medium in one liter of purified water.
2. Mix until suspension is uniform. Heat to dissolve the medium completely.
3. Dispense and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. AVOID OVERHEATING.
4. Cool to 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.
5. If EMB Agar is inoculated on the same day, it may be used without autoclave sterilization.

Quality Control Specifications

Dehydrated Appearance	Light Pink-Purple, homogeneous, free flowing powder
Solution	3.6% Solution in Distilled or deionized water is soluble on boiling, Reddish purple colored, and slightly hazy.
Prepared Medium	Reddish purple coloured, opalescent gel with greenish cast and finely dispersed precipitate forms in Petri plates
Reaction of 3.6% Solution	pH 7.2 ± 0.2 at 25°C
Gel Strength	Firm, compared to 1.35% Agar Gel.

Expected Cultural Response: Cultural response on EMB Agar observed after incubation at 35-37°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery %	Colour of colony
1.	*Enterobacter aerogenes ATCC 13048*	50-100	good	40-50%	pink, without sheen
2.	*Escherichia coli ATCC 25922*	50-100	luxuriant	>=50%	purple with black centre and green metallic sheen
3.	*Klebsiella pneumoniae ATCC 13883*	50-100	good	40-50%	pink, mucoid
4.	*Proteus mirabilis ATCC 25933*	50-100	luxuriant	>=50%	colourless
5.	*Salmonella typhimurium ATCC 14028*	50-100	luxuriant	>=50%	colourless
6.	*Staphylococcus aureus ATCC 25923*	>=10^3	inhibited	0%	--

The organisms listed are the minimum that should be used for quality control testing.

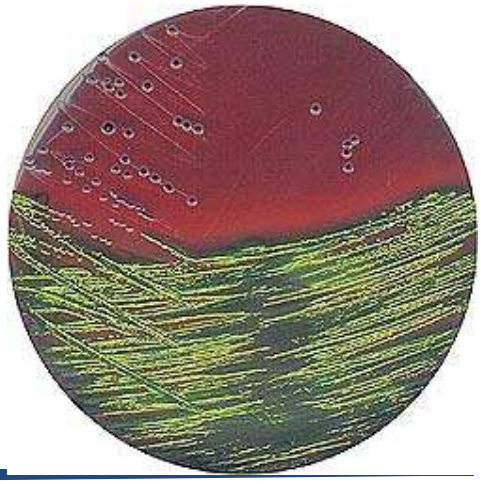
Test Procedure

1. Observe aseptic techniques.
2. Use standard procedures to obtain isolated colonies from specimens.
3. A nonselective medium should also be streaked to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen.
4. Incubate plates, protected from light, at 35 ± 2°C for 18-24 hours. If negative after 24 hours, reincubate an additional 24 hours.
5. Confirmatory tests should be further carried out for identification of isolated colonies.

Results

After 18 – 24 hours of incubation at 35 ± 2°C, Typical colonial morphology on EMB Agar is as follows:

Escherichia coli..... Large, blue-black, green metallic sheen
Enterobacter/Klebsiella..... Large, mucoid, blue-black
Proteus..... Large, colorless
Salmonella..... Large, colorless to amber
Shigella..... Large, colorless to amber
Pseudomonas..... Irregular, colorless
Gram-positive bacteria..... No growth to slight growth



Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity

environment at the same storage temperature. Protect from moisture and light.

Expiration
Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure
1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Biochemical and serological tests are performed for complete identification.

Packaging
Product Name : EMB Agar
Product Code : DM092
Available Pack sizes : 100gm / 500gm

References

1. Holt-Harris, J.E., and O. Teague. 1916. A new culture medium for the isolation of Bacillus typhosus from stools. J. Inf. Dis. 18:596-600.
2. Levine, M. 1918. Differentiation of B. coli and B. aerogenes on a simplified eosin-methylene blue agar. J. Inf. Dis. 23:43-47.
3. Howard B.J., 1994, Clinical and Pathogenic Microbiology, 2nd ed., Mosby Year Book, Inc.
4. Greenberg A. E., Trussell R. R. and Clesceri L. S. (Eds.), 1998, Standard Methods ,, for the Examination of Water and Wastewater, 20th ed., APHA, Washington, D.C.
5. Marshall R. (Ed.), 1992, Standard Methods for the Examination of Dairy ,, Products, 16th ed., APHA Inc., New York.
6. Downes F. P and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.

Further Information
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