PRODUCT SPECIFICATION SHEET

Eosin Methylene Blue Agar Base (DM093)

Intended Use

Eosin Methylene Blue Agar Base / EMB Agar Base (DM093) is a basal medium to which different carbohydrates and other test substances may be added for differentiation and study of various enteric bacteria.

Product Summary and Explanation

Levine EMB Agar was developed by Levine [1,2] and is used for the differentiation of *Escherichia coli* and *Enterobacter aerogenes* and also for the rapid identification of *Candida albicans*. This medium is recommended for the detection, enumeration and differentiation of members of the coliform group by American Public Health Association [3, 4, 5]. Some gram-positive bacteria such as faecal Streptococci, yeasts grow on this medium and form pinpoint colonies. EMB Agar Base is a modification of EMB Agar, Levine without lactose. This facilitates the use of the medium as a basal agar to which desired carbohydrates could be added to differentiate between various enteric bacteria.

Principles of the Procedure

Peptic digest of animal tissue provides carbon, nitrogen, and other essential growth nutrients in the medium. Eosin-Y and methylene blue serve as differential indicators. Phosphate buffers the medium. Eosin-Y and methylene blue make the medium slightly selective and inhibit certain gram-positive bacteria. These dyes differentiate between lactose fermenters and non-fermenters. The ratio of eosin and methylene blue is adjusted approximately to 6:1. 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[3]. 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.

Formula / Liter

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.00
Dipotassium Phosphate	2.00
Eosin Y	0.40
Methylene Blue	0.065
Agar	15.00
Final pH: 7.3 ± 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.
5. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 27.5 g of the medium in one liter of purified water. Add desired carbohydrate in desired concentration before sterilization.
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 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.
5. If EMB Agar is inoculated on the same day, it may be used without autoclave sterilization.
6. Test samples of the finished product for performance using stable, typical control cultures.
7. Store the medium away from light to avoid photooxidation

Quality Control Specifications

Dehydrated Appearance	Light Pink-Purple, homogeneous, free flowing powder
Solution	2.75% 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 2.75% Solution	pH 7.3 ± 0.2 at 25°C
Gel Strength	Firm, compared to 1.5% Agar Gel.

Expected Cultural Response: Cultural response on EMB Agar Base with added carbohydrates, observed after incubation at 35 ± 2°C for 18-24 hours. Fungal cultures incubated at 25-30°C for 24-48 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-red, without sheen
2.	*Escherichia coli* ATCC 25922	50-100	luxuriant	>=50%	blue-black with metallic sheen
3.	*Candida albicans ATCC 10231*	50-100	luxuriant (incubated in 10% CO2)	>=50%	colourless
4.	*Enterococcus faecalis ATCC 29212*	50-100	non-poor	<=10%	colourless
5.	*Pseudomonas aeruginosa ATCC 27853*	50-100	luxuriant	>=50%	colourless
6.	*Salmonella Typhimurium ATCC 14028*	50-100	luxuriant	>=50%	colourless
7.	*Saccharomyces cerevisiae ATCC 9763*	50-100	non-poor	<=10%	cream
8.	*Staphylococcus aureus ATCC 25923*	50-100	non-poor	<=10%	colourless

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 2 - 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.

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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 Base
Product Code : DM093
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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