

PRODUCT SPECIFICATION SHEET

Levine Eosin-Methylene Blue Agar Medium (DM094I)

Intended Use

Levine Eosin-Methylene Blue Agar Medium (DM094I) is recommended for isolation, enumeration and differentiation of members of *Enterobacteriaceae* in compliance with IP.

Product Summary and Explanation

Salmonella Differential Agar modified is a slight modification of original formulation of Rambach (1) used for differentiation of *Salmonella* species from *Proteus* species and other enteric bacteria. Production of acid from propylene glycol is a novel characteristic of *Salmonella* species and is utilised in this medium. Many of the media such as SS Agar, XLD Agar recommended for the identification and differentiation of salmonella species (2) are based on lactose fermentation and hydrogen sulphide production. Peptone special and yeast extract supports the luxuriant growth of bacteria while sodium deoxycholate inhibits gram-positive organisms rendering the medium selective for enteric microorganisms. Sodium chloride maintains atmospheric balance. The BC indicator turns pink in presence of acid produced from propylene glycol. Lactose fermenting ability is determined by using an indicator which can detect the presence of enzyme β -galactosidase. Lactose fermenting (β -galactosidase producing) bacteria yield blue violet coloured colony (3). *Salmonellae* produce acid from propylene glycol and then after combining with the pH indicator give typical pink red colonies. Other enteric gram-negative bacteria form colourless colonies. *Salmonellae Typhimurium* and *Salmonellae Enteritidis* produce pink to red colonies.

Principles of the Procedure

Salmonella Differential Agar, Modified contains peptone special and yeast extract which supports the luxuriant growth of bacteria. Sodium deoxycholate helps to inhibit gram-positive organisms making the medium selective for enteric microorganisms. Sodium chloride maintains the osmotic balance of the medium. The BC indicator turns pink in presence of acid produced from propylene glycol. Lactose fermenting ability is determined by using an indicator which can detect the presence of enzyme β -galactosidase.

Formula / Liter

Ingredients	Gms / Liter
Pancreatic digest of gelatine	10.00
Dibasic potassium phosphate	2.00
Lactose	10.00
Eosin - Y	0.40
Methylene blue	0.065
Agar	15.00
Final pH: 7.1 ± 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. Irritating to eyes, respiratory system, and skin.
3. Store the medium away from light to avoid photooxidation.

Directions

1. Suspend 37.46 grams of medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. AVOID OVERHEATING.
5. Cool to 50°C and shake the medium in order to oxidize the methylene blue (i.e. restore its blue colour) and to suspend the precipitate, which is an essential part of the medium.
6. Mix well before pouring into sterile Petri plates.

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Quality Control Specifications

Dehydrated Appearance	Light pink to purple homogeneous free flowing powder
Prepared Medium	Reddish purple with greenish cast coloured opalescent gel with finely dispersed precipitate forms in Petri plates.
Reaction of 4.55% Solution	Not Applicable
Gel Strength	Firm, comparable with 1.5% agar gel.

Expected Cultural Response: Growth Promotion is carried out in accordance with USP. Cultural response was observed after an incubation at 30-35°C for 24-48 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar and fungal growth on Sabouraud Dextrose Agar

Sr. No.	Organisms	Results to be achieved					Incubation period
		Inoculum (CFU)	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation Temperature	
	Test for specified microorganism						
1.	<i>Escherichia coli</i> ATCC 8739	50 - 100	25 -100	>=50 %	blue-black colonies with metallic sheen	30 -35 °C	24 -48 hrs
	Additional Microbiological testing						
2.	<i>Escherichia coli</i> NCTC 9002	50 - 100	25 -100	>=50 %	blue-black colonies with metallic sheen	30 -35 °C	24 -48 hrs
3.	<i>Enterobacter aerogenes</i> ATCC 13048	50 - 100	25 -100	>=50 %	pink to red	30 -35 °C	24 -48 hrs
4.	<i>Salmonella Typhimurium</i> ATCC 14028	50 - 100	25 -100	>=50 %	colourless	30 -35 °C	24 -48 hrs
5.	<i>Pseudomonas aeruginosa</i> ATCC 9027	50 - 100	25 -100	>=50 %	colourless	30 -35 °C	24 -48 hrs
6.	<i>Enterococcus faecalis</i> ATCC 29212	>=10 ³	0	0 %	--	30 -35 °C	24 -48 hrs
7.	<i>Staphylococcus aureus</i> ATCC 6538	>=10 ³	0	0 %	--	30 -35 °C	24 -48 hrs
8.	<i>Candida albicans</i> ATCC 10231	50 -100	25 -100	>=50 %	colourless	30 -35 °C	24 -48 hrs
9.	<i>Saccharomyces cerevisiae</i> ATCC 9763	50 -100	0 -10	0 -10 %	cream	30 -35 °C	24 -48 hrs

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

Test Procedure

Refer to appropriate references for standard test procedures.

Results

- Lactose fermenting (β -galactosidase producing) bacteria yield blue violet coloured colony.
- Salmonellae* produce acid from propylene glycol and which on combining with the pH indicator gives typical pink red colonies.
- Other enteric gram-negative bacteria form colourless colonies.
- Salmonella typhimurium* and *Salmonella enteritidis* produce pink to red colonies.



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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.

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Levine Eosin-Methylene Blue Agar Medium

Product Code : DM094I

Available Pack sizes : 500gm

References

1. Rambach A., 1990, Environ. Microbiol., 56:301.
2. Eaton A. D., Clesceri L. S., Rice E. W. and Greenberg A W., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st Ed., APHA, Washington, D.C
3. Greenwald R., Henderson R.W. and Yappaw S., 1991, J. Clin. Microbiol., 29:2354.

Further Information

For further information please contact your local MICROMASTER Representative.



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