Technical Data Sheet

GranuCult® prime

LEVINE Eosin-Methylene blue (L-EMB) agar acc. ISO 21150 and FDA-BAM

Ordering number: 1.03857.0500

For the isolation, confirmation and identification of *Escherichia coli* and other *Enterobacteriaceae* from the food chain, cosmetic and other materials.

LEVINE Eosin-Methylene blue (L-EMB) agar is also known as Eosin Methylene-blue Lactose Agar acc. to LEVINE or EMB agar, LEVINE.

This culture medium complies with the specifications given by EN ISO 21150, FDA-BAM Medium M80, GB 4789.6 and with APHA.

This culture medium is released by the quality control laboratory of Merck KGaA, Darmstadt, Germany. The laboratory is accredited by the German accreditation authority DAkkS as registered test laboratory D-PL-15185-01-00 according to DIN EN ISO/IEC 17025 for the performance testing of media for microbiology according to DIN EN ISO 11133.

Mode of Action

This culture medium is a low selective and differential medium containing lactose and two indicator dyes, Eosin Y and Methylene Blue. The use of these two indicators allows differentiation between colonies of lactose fermenting and non-fermenting organisms.

The peptones provide nitrogen, minerals and amino acids, and lactose provide the carbon sources. Di-potassium phosphate acts as a buffer whilst Eosin yellowish (Eosin Y) and methylene blue serve as differential indicators and inhibitors. Eosin inhibits the most Gram-positive bacteria. Agar is the solidifying agent.

Coliforms produce blue-black colonies due to the incorporation of an eosin-methylene blue dye complex when the pH drops down. This causes the formation of an amide bonding between the eosin and methylene blue. Colonies of *Escherichia coli* may show a characteristic green metallic luster due to the high acid production from lactose, which causes the distinctive metallic sheen of *E. coli* on this medium. Other coliforms do not produce enough acid to cause this reaction.

Lactose non-fermenting bacteria (like most *Salmonella* and *Shigella*) will form colorless or transparent amber coloured colonies, but some strains of *Salmonella* and *Shigella* may not grow on this culture medium. Some Gram-positive bacteria and yeasts may grow on this culture medium and usually form pinpoint colonies.

Typical Composition

APHA specifies no composition for LEVINE Eosin-Methylene blue (L-EMB) agar.

Specified by EN ISO 21150		**Specified by FDA-BAM Medium M80, GB 4789.6**		**GranuCult® prime LEVINE Eosin-Methylene blue (L-EMB) agar acc. ISO 21150 and FDA-BAM**	
Pancreatic digest of gelatin	10.0 g/l	Peptone	10.0 g/l	Pancreatic digest of gelatin	10.0 g/l
Lactose	10.0 g/l	Lactose	10.0 g/l	Lactose	10.0 g/l
K_2HPO_4	2.0 g/l	K_2HPO_4	2.0 g/l	K_2HPO_4	2.0 g/l
Eosin Y	0.4 g/l	Eosin Y	0.4 g/l	Eosin yellowish (Eosin Y)	0.4 g/l
Methylene blue	0.065 g/l	Methylene blue	0.065 g/l	Methylene blue	0.065 g/l
Agar	13.5 g/l	Agar	15.0 g/l	Agar-agar*	13.5 g/l
Water	1000 ml	Water	1000 ml	Water	n/a
pH at 25 °C	7.1 ± 0.2	pH at 25 °C	7.1 ± 0.2	pH at 25 °C	7.1 ± 0.2

*Agar-Agar is equivalent to other different terms of agar.

Preparation

Dissolve 36.0 g in 1 liter of purified water. Heat in boiling water and agitate frequently until completely dissolved. Autoclave (15 minutes at 121 °C). Pour to plates.

The dehydrated medium is a granulate with violet color.

The prepared medium is clear to slightly opalescent and re-brown. The pH value at 25 °C is in the range of 6.9 - 7.3.

Before inoculation, allow the prepared medium to equilibrate at room temperature if it was stored at a lower temperature.

There should be no visible moisture on the plates before use. When moisture is present, the plates should be dried for the minimum time required to remove visible moisture, following the procedure as described by EN ISO 11133.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Following the procedure given by EN ISO 21150, APHA, FDA-BAM Chapter 4 and 4A, GB 4789.6 and inoculate the surface of the medium from the selective enriched cultures or from the isolation medium so that well-isolated colonies will be obtained.

Incubate the inoculated plates inverted under aerobic conditions, e.g.

- acc. to EN ISO 21150 at (32,5 ± 2,5 °C) for at least 24 h (maximum 48 h);
- acc. to FDA-BAM Chapter No. 4 and Chapter No. 4A at (35 ± 0.5 °C) for 18-24 h;
- acc. to GB 4789.6 at (36 ± 1 °C) for between for 18 h to 24 h.

On LEVINE Eosin-Methylene blue (L-EMB) agar acc. ISO 21150, FDA-BAM and GB 4789.6, *Escherichia coli* produce flat colonies mostly with a metallic sheen under reflected light and a black-blue appearance and a dark center under transmitted light.
Lactose non-fermenting bacteria produce colorless colonies without a metallic sheen under reflected light and without a blue-black appearance under transmitted light.

Colonies of the most important bacteria usually have the appearance described below:

Appearance of colonies	**Microorganisms**
Diameter 2-3 mm, greenish metallic sheen in reflected light, dark or even black-blue centre in transmitted light	*Escherichia coli*
Diameter 4-6 mm, grey-brown centre in transmitted light, no metallic sheen	*Enterobacter*
Transparent, amber-coloured	*Salmonella* and *Shigella*
Colorless, "pin-point" colonies (if growth)	Gram-positive bacteria

This presumptive evidence must be confirmed by carrying out the usual tests.

Storage

Store at +15 °C to +25 °C, dry and tightly closed. Do not use clumped or discolored medium. Protect from UV light (including sun light). For *in vitro* use only.

Acc. to MacFaddin (1985), self-prepared plates can be stored in the dark and protected against evaporation at (5 ± 3 °C) for 6-8 weeks.

Microbiological Performance

The performance test is in accordance with the current version of EN ISO 11133 and EP/JP/USP.

Test method: Performance testing of solid culture media - Qualitative methods

Qualitative method for solid media		
Test strain	**Specification**	
	Growth	**Typical reaction**
Escherichia coli ATCC® 8739 [WDCM 00012]	good	colonies with metallic sheen under reflected light and a blue-black appearance with a dark or even black center under transmitted light
Escherichia coli ATCC® 25922 [WDCM 00013]	good	colonies with metallic sheen under reflected light and a blue-black appearance with a dark or even black center under transmitted light
Enterobacter cloacae ATCC® 13047 [WDCM 00083]	good	colonies without metallic sheen under reflected light and without a blue-black appearance under transmitted light
Salmonella Typhimurium ATCC® 14028 [WDCM 00031]	good	colonies without metallic sheen under reflected light and without a blue-black appearance under transmitted light
Staphylococcus aureus ATCC® 25923 [WDCM 00034]	no growth or partial inhibition	if growth: colourless “pin-point” colonies

Incubation: 22 ± 2 h at 35 ± 1 °C, aerobic.

Please refer to the actual batch related Certificate of Analysis.

Literature

APHA (2018): Part 9260: Detection of pathogenic bacteria. Standard Methods for the Examination of Water. 23rd ed. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, D.C.

APHA (2015) Chapter No. 60: Bottled water. and Chapter No. 67: Microbiological media, reagents and stains. Compendium of Methods for the Microbiological Examination of Foods. 5th ed. American Public Health Association, Washington, D.C.

APHA (2004) Chapter 7: Coliform and other indicator bacteria. Standard Methods for the Examination of Dairy Products. 17th ed. American Public Health Association, Washington, D.C.

EN ISO International Standardisation Organisation. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media + Amendment 1 + Amendment 2. EN ISO 11133:2014/Amd1:2018/Amd2:2020.

EN ISO International Standardisation Organisation. Cosmetics — Microbiology — Detection of *Escherichia coli*. EN ISO 21150:2015.

FDA-BAM (2020): Chapter No. 4: Enumeration of *Escherichia coli* and the Coliform Bacteria. Food and Drug Administration - Bacteriological Analytical Manual.

FDA-BAM (2020): Chapter No. 4A: Diarrheagenic *Escherichia coli* Food and Drug Administration - Bacteriological Analytical Manual.

FDA-BAM (2018): Media Index for BAM - BAM Media M80: Levine's Eosin-Methylene Blue (L-EMB) Agar. Food and Drug Administration - Bacteriological Analytical Manual.

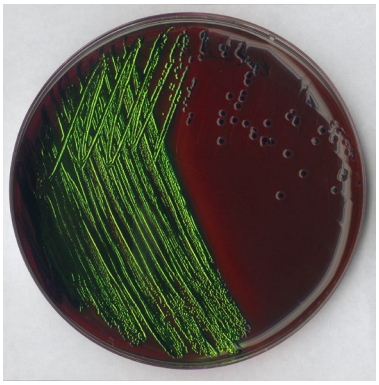
National Health and Family Planning Commission of the People's Republic of China. China Food and Drug Administration. National Standard of the People's Republic of China. National food safety standard — Food microbiological examination: Examination of diarrheagenic *Escherichia coli*. GB 4789.6-2016.

Girolami, R.L. and Stamm, J.M. (1976): Inhibitory effect of light on growth-supporting properties of eosin methylene blue agar. **31**: 141-142.

Levine, M. (1918): Differentiation of *E. coli* and *B. aerogenes* on a simplified eosin-methylene blue agar. J. Infect. Dis. **23**: 43-47.

Manafi, M. (2012): Culture media for the detection and enumeration of "total" *Enterobacteriaceae*, coliforms and *Escherichia coli* from foods. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds). pp. 233-260. Royal Society of Chemistry, Cambridge, UK.

McFaddin J.F. (1985): Eosin Methylene Blue Agars. In: Media for isolation – cultivation – identification – maintenance of medical bacteria. Volume I. pp. 292-297. Lippincott Williams and Wilkins, Baltimore, MD, USA.



Escherichia coli
ATCC® 25922
[WDCM 00013]

Salmonella Typhimurium
ATCC® 14028
[WDCM 00031]

Enterobacter cloacae
ATCC® 13047
[WDCM 00083]

Ordering Information

Product	Cat. No.	Pack size
GranuCult® prime LEVINE Eosin-Methylene blue (L-EMB) agar acc. ISO 21150 and FDA-BAM	1.03857.0500	500 g
GranuCult® plus EMB (Eosin-Methylene blue) agar acc. HOLT-HARRIS and TEAGUE	1.03858.0500	500 g