

Technical Data Sheet

GranuCult® plus EMB (Eosin-Methylene blue) agar acc. HOLT-HARRIS and TEAGUE

Ordering number: 1.03858.0500

For the isolation and differentiation of *Escherichia coli* and other Gram-negative bacteria from different materials.

EMB (Eosin-Methylene blue) agar acc. HOLT-HARRIS and TEAGUE is also known as Holt-Harris & Teague (HHT) EMB agar, as Eosin Methylene Blue Lactose Sucrose agar and as Eosin Methylene Blue agar, modified.

Mode of Action

This culture medium is a low selective and differential medium containing lactose and sucrose and two indicator dyes, Eosin Y and Methylene Blue. The use of these two indicators allows differentiation between colonies of lactose and/or sucrose fermenting and non-fermenting organisms. Sucrose is included since certain members of the coliform group of microorganisms ferment sucrose more readily than lactose. This formulation does not discriminate between carbohydrate utilization (lactose or sucrose).

The peptones provide nitrogen, minerals and amino acids, lactose and sucrose 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. 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. Colonies of *Escherichia coli* may show a characteristic green metallic luster due to the rapid fermentation of lactose. Lactose- and sucrose-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

GranuCult® plus EMB (Eosin-Methylene blue) agar acc. HOLT-HARRIS and TEAGUE	
Peptones	10.0 g/l
K ₂ HPO ₄	2.0 g/l
Lactose	5.0 g/l
Sucrose	5.0 g/l
Eosin yellowish (Eosin Y)	0.4 g/l
Methylene blue	0.07 g/l
Agar-agar*	13.5 g/l
Water	n/a
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 pink color.

The prepared medium is clear to slightly opalescent and reddish-brown to violet-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.

Inoculate by spreading or streaking to permit discrete colonies.

Incubate 24 to 48 h, e. g. at (35 ± 1) °C, aerobic.

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

Appearance of colonies	Microorganisms
Translucent, amber coloured	Lactose- and sucrose-negative: <i>Salmonella</i> spp., <i>Shigella</i> spp. and others.
Greenish, metallic luster in reflected light, blue-black center in transmitted light	mostly <i>Escherichia coli</i>
Mucoid, confluent, gray-brown centered colonies in transmitted light, often larger than those of <i>E. coli</i>	<i>Enterobacter</i> spp., <i>Klebsiella</i> spp. and others

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.

Microbiological Performance

Test strain	Specification	
	Growth	Typical reaction
<i>Escherichia coli</i> ATCC® 25922 [WDCM 00013]	Good to very good	Metallic luster
<i>Escherichia coli</i> ATCC® 8739 [WDCM 00012]	Good to very good	Metallic luster
<i>Salmonella</i> Typhimurium ATCC® 14028 [WDCM 00031]	Good to very good	No metallic luster
<i>Bacillus cereus</i> ATCC® 11778 [WDCM 00001]	None to poor	No metallic luster

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

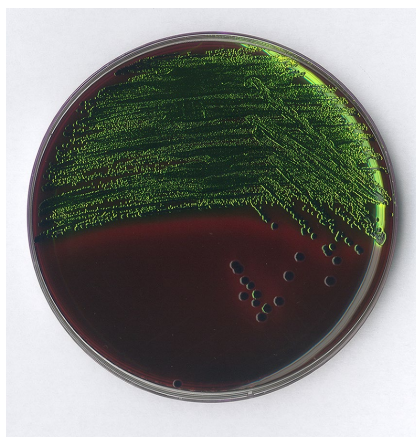
Please refer to the actual batch related Certificate of Analysis.

Literature

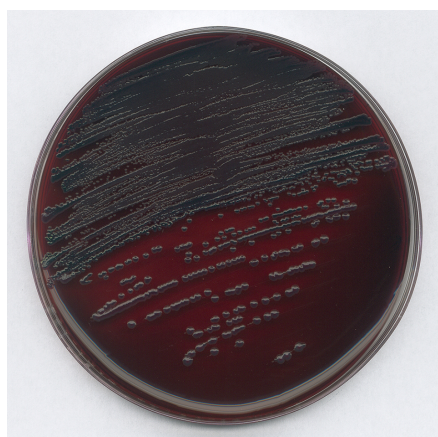
EN ISO International Standardisation Organisation. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media + Amendment 1 + Amendment 2. EN ISO 11133:2014/Amd1:2018/Amd2:2020.

Holt-Harris, J.E. and Teague, O.A. (1916). A new culture medium for the isolation of *Bacillus typhosus* from stools. J. Infect. Dis. **18**: 596-600.

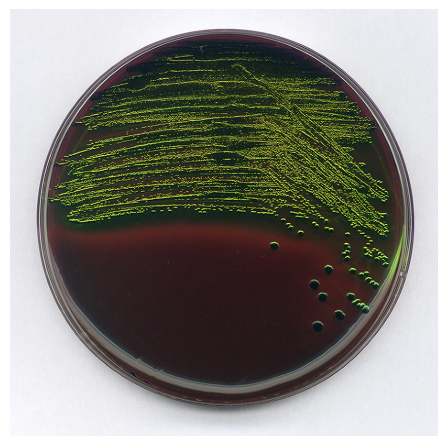
McFaddin J.F. (1985): Eosin Methylene Blue Agars. In: Media for isolation – cultivation – identification – maintenance of medical bacteria. Volume I. pp. 292-297. Lippincott Williams and Wilkins, Baltimore, MD, USA.



Escherichia coli
ATCC® 25922
[WDCM 00013]



Enterobacter cloacae
ATCC® 13047
[WDCM 00083]



Escherichia coli
ATCC® 8739
[WDCM 00012]

Ordering Information

Product	Cat. No.	Pack size
GranuCult® plus EMB (Eosin-Methylene blue) agar acc. HOLT-HARRIS and TEAGUE	1.03858.0500	500 g

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