

Instructions for Use

M ENDO LES AGAR

Cat. no. G28	m Endo LES Agar, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. G128	m Endo LES Agar, 15x60mm Plate, 11ml	10 plates/bag

INTENDED USE

Hardy Diagnostics' m Endo LES Agar is recommended for use in enumerating coliforms in water by the single-step, two-step and delayed incubation membrane filtration methods.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

Hardy Diagnostics' m Endo LES Agar follows the Lawrence Experimental Station (LES) formula developed by McCarthy, et al.^(1,2) Through the course of their studies, McCarthy and colleagues employed the use of lauryl sulfate as a primary enrichment broth and followed a two membrane filtration technique as opposed to the most probable number (MPN) method or one-step method. The researchers found that use of a two-step process of enrichment resulted in higher recovery of coliforms and more reliable and precise results.^(1,2)

m Endo LES Agar contains deoxycholate and lauryl sulfate, which serve as inhibitory agents against gram-positive microorganisms; lactose, which is a source of fermentable carbohydrate; peptones and yeast extract, which provide necessary growth nutrients; basic fuchsin acts as the pH indicator.

Microorganisms capable of lactose-fermentation produce acetaldehyde, which reacts with basic fuchsin and sodium sulfite to form a red zone surrounding the colonies. Coliform organisms produce red colonies with a characteristic golden-green metallic sheen. The development of a metallic sheen occurs when the organism produces aldehydes during the rapid fermentation of lactose. If the inoculum is too heavy, the sheen will be suppressed. Bacteria unable to ferment lactose form clear, colorless colonies.

The American Public Health Association specifies using m Endo LES Agar in the standard total coliform membrane filtration procedure for testing drinking and bottled water.^(3,4) It is also specified for use in the completed phase of the standard total coliform fermentation technique.⁽³⁾ The U.S. Environmental Protection Agency specifies using m Endo LES Agar in the total coliform methods for testing water using single-step, two-step and delayed incubation membrane filtration methods.^(5,6)

FORMULA

Ingredients per liter of deionized water:*

Lactose	9.4gm
Pancreatic Digest of Casein	7.5gm

Peptic Digest of Animal Tissue	7.5gm
Sodium Chloride	3.7gm
Dipotassium Phosphate	3.3gm
Sodium Sulfite	1.6gm
Yeast Extract	1.2gm
Monopotassium Phosphate	1.0gm
Basic Fuchsin	0.8gm
Sodium Deoxycholate	0.1gm
Sodium Lauryl Sulfate	0.05gm
Ethanol 95%	20.0ml
Agar	15.0gm

Final pH 7.2 +/- 0.2 at 25°C.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt, store the product at 2-8°C. Products should not be used if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "[Storage](#)" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "[Guidelines for Isolation Precautions](#)" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

PROCEDURE

Sample collection: Consult listed references for information on sample collection.⁽³⁻⁶⁾

Single-Step Method:

1. Filter the water sample through a 0.45um membrane filter.
2. Place the membrane on the surface of the agar, filtered side up, using a rolling motion to assure proper contact with the surface and to avoid entrapment of air bubbles.
3. Incubate plates at 35 +/- 0.5°C. for 24 +/- 2.0 hours.
4. Observe for the presence of red colonies with a golden-green metallic sheen.

Two-Step Method:

1. Invert a plate of m Endo LES Agar and insert an absorbent filter pad in the lid of the plate.
2. Pipet 1.8 to 2.2ml of Lauryl Sulfate Broth (Cat. no. K33) to the filter pad. Remove excess fluid.
3. Run the water sample through the membrane filter.
4. With the top side of the filter up, apply a membrane filter, through which a water sample has been filtered, to the membrane filter pad. Apply the membrane filter using a rolling motion to avoid entrapment of air bubbles.
5. Incubate, in a humid (at least 60%) atmosphere, at 35 +/- 0.5°C. for a period of 2 hours +/- 0.5 hours.
6. Following incubation, strip the membrane filter from the pad and, using a rolling motion, place the filter face up on the surface of the m Endo LES Agar.
7. Reincubate the plate at 35 +/- 0.5°C. for 24 hours.
8. Observe for the presence of red colonies with a golden-green metallic sheen.

INTERPRETATION OF RESULTS

Red to red-black colonies with a golden-green metallic sheen are indicative of coliform microorganisms. All such colonies should be counted and reported as total coliform count per volume of water sampled. Dilution factors must be taken into account.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Variations in degree of metallic sheen development may be observed among coliform strains.⁽³⁾

Refer to the document "[Limitations of Procedures and Warranty](#)" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following

microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Escherichia coli</i> ** ATCC® 25922	MF	24hr	35°C	Aerobic	Growth; red to red-black colonies with a metallic sheen
<i>Salmonella enterica</i> ATCC® 14028	MF	24hr	35°C	Aerobic	Growth; colorless colonies
<i>Staphylococcus aureus</i> ** ATCC® 25923	B	24hr	35°C	Aerobic	Inhibited

* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

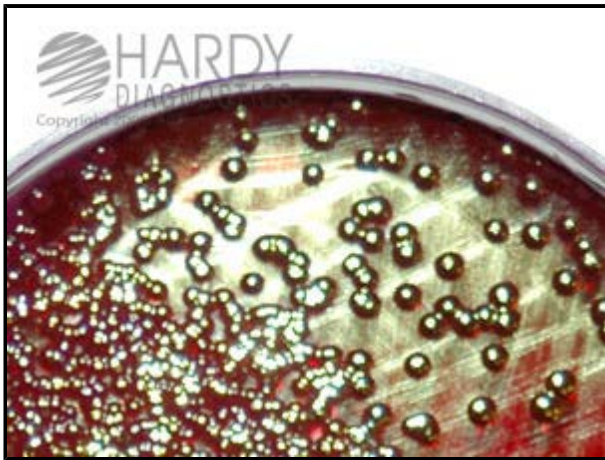
** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CoFA) available from Hardy Diagnostics [Certificate of Analysis](#) website. Also refer to the document "[Finished Product Quality Control Procedures](#)," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

m Endo LES Agar should appear opalescent, with a slight precipitate throughout, and light rose in color.



Escherichia coli (ATCC® 25922) colonies growing on m Endo LES Agar (Cat. no. G28). Incubated aerobically for 24 hours at 35°C.



Salmonella enterica (ATCC® 14028) colonies growing on m Endo LES Agar (Cat. no. G28). Incubated aerobically for 24 hours at 35°C.



Staphylococcus aureus (ATCC® 25923) growth inhibited on m
Endo LES Agar (Cat. no. G28). Incubated aerobically for 24 hours
at 35°C.

REFERENCES

1. McCarthy, J.A., et al. 1961. *Water Sewage Works*; 108:238-243.
2. McCarthy, J.A., et al. 1958. *AJPH*; 48:16-28.
3. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*, APHA, Washington, D.C.
4. Cowman, S. and R. Kelsey. 1992. *Compendium of Methods for the Microbiological Examination of Foods*, 3rd ed. American Public Health Association, Washington, D.C.
5. Bordner, R. and J. Winter. 1978. *Microbiological Methods for Monitoring the Environment, Water and Wastes*, EPA-600/8-78-017. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH.
6. Environmental Protection Agency. *Manual for the Certification of Laboratories Analyzing Drinking Water*, EPA-814B-92-002. Office of Ground Water and Technical Support Division, U.S. Environmental Protection Agency, Cincinnati, OH, 1992.

ATCC is a registered trademark of the American Type Culture Collection.

IFU-10857[B]



1430 West McCoy Lane, Santa Maria, CA 93455, USA

Phone: (805) 346-2766 ext. 5658

Fax: (805) 346-2760

Website: HardyDiagnostics.com

Email: TechnicalServices@HardyDiagnostics.com

[Ordering Information](#)

Distribution Centers:

California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

Copyright© 2020 by Hardy Diagnostics. All rights reserved.

HDQA 2207D [D]