

# Instructions for Use

## CRITERION™ M ENDO LES AGAR

<a href="#">Cat. no. C7410</a>	CRITERION™ m Endo LES Agar	102gm
<a href="#">Cat. no. C7411</a>	CRITERION™ m Endo LES Agar	500gm
<a href="#">Cat. no. C7412</a>	CRITERION™ m Endo LES Agar	2kg
<a href="#">Cat. no. C7413</a>	CRITERION™ m Endo LES Agar	10kg
Cat. no. C7414	CRITERION™ m Endo LES Agar	50kg

## INTENDED USE

Hardy Diagnostics' CRITERION™ 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 dehydrated culture medium is a raw material intended to be used in the making of prepared media products, which will require further processing, additional ingredients, or supplements.

## SUMMARY

CRITERION™ m Endo LES Agar follows the Lawrence Experimental Station (LES) formula developed by McCarthy, et al.<sup>(1,2)</sup> 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.<sup>(1,2)</sup>

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; and 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.<sup>(3,4)</sup> It is also specified for use in the completed phase of the standard total coliform fermentation technique.<sup>(3)</sup> 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.<sup>(5,6)</sup>

## FORMULA\*

Gram weight per liter:	51.0gm/L
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
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

Store the sealed bottle(s) containing dehydrated culture medium at 2-30°C. Dehydrated culture medium is very hygroscopic. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. The dehydrated culture media should be discarded if it is not free-flowing or if the color has changed from its original purple.

Store the prepared media at 2-8°C.

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.

**Warning:** Basic Fuchsin is a potential carcinogen and care must be taken to avoid contamination of the skin. If contact occurs, rinse thoroughly with water.

## METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Suspend 51.0gm of the dehydrated culture media in 1 liter of distilled or deionized water.
2. Add 20ml of ethanol (95%, not denatured).
3. Heat to 97.5 - 98.5°C. **Do not allow to boil. Do not autoclave.**
4. Cool to 50-55°C. and dispense approximately 5-7ml into sterile 60mm petri dishes.
5. **Protect from light.** This product is extremely light sensitive.

## PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed references or refer to the prepared media Instructions for Use (IFU) for Cat. No. G28.

## LIMITATIONS

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

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results. Always take pH reading at room temperature.

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

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

## MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as autoclaves incinerators, and incubators, etc., 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>	MF	24hr	35°C	Aerobic	Growth; colorless colonies

ATCC® 14028					
<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

Users of dehydrated 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. In addition, refer to the following document "[Finished Product Quality Control Procedures](#)," for more information on QC or see the reference(s) for more specific information.

## PHYSICAL APPEARANCE

CRITERION™ m Endo LES Agar powder should appear homogeneous, free-flowing, and purple in color. The prepared media should appear opalescent with a slight precipitate throughout, and light rose in color.

## 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. *Standard Methods for the Examination of Water and Wastewater*, 19th ed. 1995. American Public Health Association, 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-10301[A]



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