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Instructions for Use

CRITERION™ ESCULIN MANNITOL AGAR

Cat. no. C7590	CRITERION™ Esculin Mannitol Agar	108gm
Cat. no. C7591	CRITERION™ Esculin Mannitol Agar	500gm
Cat. no. C7592	CRITERION™ Esculin Mannitol Agar	2kg
Cat. no. C7593	CRITERION™ Esculin Mannitol Agar	10kg
Cat. no. C7594	CRITERION™ Esculin Mannitol Agar	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Esculin Mannitol Agar is a dual purpose medium. It is recommended for use as a differential medium for the presumptive identification of enterococci, and a selective and differential medium for the isolation of *Staphylococcus aureus*. This media is particularly useful for the testing of dairy foods.

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

Because enterococci have a higher survival rate than coliforms in adverse environments, such as salted butter and the low pH of yogurt, they are generally better index organisms for the sanitary conditions of dairy products *Staphylococcus aureus* can be found in raw milk, dry milk, cheese and ice cream. CRITERION™ Esculin Mannitol Agar is helpful in recovering these pathogens.[7]

This medium contains esculin and ferric citrate. When esculin is hydrolyzed by group D streptococci it becomes esculetin. Esculetin reacts with the ferric ions from the ferric citrate and turns the medium from its original amber color to a dark brown or black. This blackening of the agar is a presumptive identification for group D enterococci.[3]

Staphylococcus aureus, which grows well on this agar, ferments mannitol. Non-pathogenic staphylococci usually show less luxuriant growth on this medium and do not ferment mannitol. Phenol red acts as a pH indicator. When mannitol is fermented, acid is produced which causes a color change in the agar from red to yellow. Those staphylococci that do not ferment mannitol show a purple or red zone around the colonies.[5] The beef extract and peptones supply the essential carbon, nitrogen and sulfur.

FORMULA

Gram weight per liter:	54.0gm/L
Pancreatic Digest of Casein	10.0gm
Mannitol	10.0gm

Yeast Extract	5.0gm
Peptic Digest of Animal Tissue	5.0gm
Sodium Chloride	5.0gm
Brain Heart Infusion	3.0gm
Corn Starch	1.0gm
Esculin	1.0gm
Ferric Ammonium Citrate	0.5gm
Phenol Red	25.0mg
Nalidixic Acid	15.0mg
Colistin Sulfate	10.0mg
Agar	13.5gm

Final pH 7.3 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-30ºC. 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 pinkish-beige.

Store the prepared culture media at 2-8ºC.

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.

METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Suspend 54.0gm of the dehydrated culture media in 1 liter of distilled or deionized water. Stir to mix thoroughly.

2. Heat to boiling to dissolve completely.

3. Sterilize in the autoclave at 121ºC. for 15 minutes.

4. Cool to 45-50ºC.

PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed references.

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.

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	
Staphylococcus aureus ATCC® 25923	A	18-48 hr	35°C	Aerobic	Growth; media turns yellow at 24-48 hours
Enterococcus faecalis ATCC® 29212	A	18-48 hr	35°C	Aerobic	Growth; blackening of media around colonies
Proteus mirabilis ATCC® 12453	A	18-48 hr	35°C	Aerobic	Partial to complete inhibition
Pseudomonas aeruginosa ATCC® 27853	A	18-48 hr	35°C	Aerobic	Partial to complete inhibition

* Refer to the document "Inoculation Procedures for Media QC" for more information.

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™ Esculin Mannitol Agar powder should appear homogeneous, free-flowing, and pinkish-beige in color. The prepared media should appear clear, and red in color.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology,* C.V. Mosby Company, St. Louis, MO.

3. MacFaddin, J.F. *Biochemical Tests for Identification of Medical Bacteria,*, Lipincott Williams & Wilkins, Philadelphia, PA.

4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook,* Vol. I, II & III. American Society for Microbiology, Washington, D.C.

5. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria,* Vol. I. Williams & Wilkins, Baltimore, MD.

6. Jorgensen., et al. *Manual of Clinical Microbiology,* American Society for Microbiology, Washington, D.C.

7. Marshall, R.T. 1993. *Standard Methods for the Examination of Dairy Products*, 13th ed. APHA, Washington, D.C.

8. Vanderzant, C. and D.F. Splittstoesser, (ed.). 1992. *Compendium of Methods for the Microbiological Examination of Foods*, 3rd ed. APHA, Washington, D.C.

9. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology,* J.B. Lippincott Company, Philadelphia, PA.

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Ordering Information

Distribution Centers:

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