IFU

Instructions for Use

ESCULIN AGAR

Cat. no. L29	Esculin Agar, 16x100mm Tube, 6.5ml Slant	20 or 100 tubes/box

INTENDED USE

Hardy Diagnostics Esculin Agar is recommended for the detection of esculin hydrolysis by microorganisms. It is particularly useful in the differentiation of *Bacteroides* species.

SUMMARY

In 1909, Harrison and Van der Leck observed the ability of organisms to hydrolyze esculin.[6] The esculin hydrolysis test was used in 1924 by Rochaix for use in the identification of group D streptococci.[7] Smith, and later Dowell, employed its use in the differentiation of anaerobic bacteria and *Bacteroides* spp., respectively.[8,9]

Esculin Agar consists of a heart infusion basal medium supplemented with vitamin K, hemin, 0.1% esculin and ferric citrate. The nutritional growth requirements of bacteria are supplied by the heart infusion. Vitamin K and hemin are added to enhance the growth of anaerobic microorganisms. Esculin and ferric citrate allow for the detection of esculin hydrolyzing microorganisms.

Esculin, a water soluble glycoside, is hydrolyzed by certain bacteria to yield glucose and esculetin. Esculetin reacts with the ferric ions to produce a black colored complex that surrounds the colonies. Microorganisms that are not capable of hydrolyzing esculin will not produce a blackening in the medium.

FORMULA

Ingredients per liter of deionized water:*

Dipeptone	10.0gm
Pancreatic Digest of Casein	6.0gm
Sodium Chloride	5.0gm
Beef Heart Infusion	2.0gm
Yeast Extract	2.0gm
Esculin	1.0gm
Ferric Citrate	0.5gm
Vitamin K	10.0mg
Hemin	5.0mg
Agar	15.0gm

Final pH 7.0 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-30ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or 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 Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic 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

Specimen Collection: Specimen collection is not applicable since this medium is not intended for primary isolation. As a general rule, infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation. Consult listed references for information on specimen collection.[1-5]

Method of Use:

1. Using a fishtail motion, inoculate the surface of the slant with 3-4 pure colonies that are 18-24 hours old.

2. Incubate the inoculated medium aerobically or anaerobically (dependant upon the isolates growth requirements) at 35-37ºC. for 24-72 hours.

3. Observe for the development of a brownish-black to black color in the medium surrounding the colonies.

INTERPRETATION OF RESULTS

A positive esculin hydrolysis test is indicated by the development of a brownish-black to black color in the medium surrounding the colonies.

A negative esculin hydrolysis test is denoted by no color change in the medium.

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.

Iron sulfide, which appears as a sooty black color on the medium, may be produced, and thus, may lead to difficulty in test interpretation.

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	
Enterococcus faecalis ATCC® 29212	A	24-48hr	35°C	Aerobic	Growth; blackening around colonies
Escherichia coli ATCC® 25922	B	24-48hr	35°C	Aerobic	Growth; no color development in media

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

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

Esculin Agar should appear clear, and medium amber with a slight blue tinge 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.

Enterococcus faecalis (ATCC® 29212) colonies growing on Esculin Agar (Cat. no. L29). Incubated aerobically for 24 hours at 35ºC.

Escherichia coli (ATCC® 25922) colonies growing on Esculin Agar (Cat. no. L29). Incubated aerobically for 24 hours at 35ºC.

Uninoculated tube of Esculin Agar (Cat. no. L29).

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

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

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

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

6. Harrison, R.C., and Van der Leck, J. 1909. Esculin Bile Salt Media for Water Analysis, Bakt. Abt.; 222:549.

7. Rochaix, A. 1924. Cr. Soc. Biol., Paris, France; 90:771.

8. Smith, L. 1975. The Pathogenic Anaerobic Bacteria, 2nd ed. Springfield, IL.

9. D.H.E.W. Publ., CDC; 72:8272, 1974.

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HARDY DIAGNOSTICS

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:

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

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