

Instructions for Use

TSA WITH BLOOD AND 0.1% ESCULIN

Cat. no. A19BX	TSA (Tryptic Soy Agar) with Blood and 0.1% Esculin, 15x100mm Plate, 19ml	100 plates/box
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INTENDED USE

Hardy Diagnostics TSA (Tryptic Soy Agar) with Blood and 0.1% Esculin is recommended for use in screening dairy products for bacteria.

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

SUMMARY

TSA (Tryptic Soy Agar) with Blood and 0.1% Esculin is a non-selective and differential medium for the cultivation, enumeration, and differentiation of organisms based on hemolysis and esculin hydrolysis. The medium is composed of Tryptic Soy Agar as the basal medium. TSA is a general, all-purpose culture medium containing digests of soybean meal and casein. Sheep blood is added to facilitate the growth of more fastidious strains and to allow for the observation of hemolytic reactions. The absence of reducing sugars and carbohydrates allows hemolysis to occur without hindrance. In addition, esculin, a water soluble glycoside, is added to differentiate organisms capable of hydrolyzing esculin to yield glucose and esculetin, such as group D streptococci, from those that are not (e.g. *Streptococcus agalactiae*). Esculetin reacts with ferric ions to produce a greening or darkening of the medium surrounding colonies. Microorganisms incapable of hydrolyzing esculin will not produce this reaction.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	15.0g
Peptic Digest of Soybean Meal	5.0g
Sodium Chloride	5.0g
Sheep Blood	60.0ml
Esculin	1.0g
Agar	15.0g

Final pH 7.3 +/- 0.2 at 25°C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt, store at 2-8°C away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), hemolysis, 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 reference methods for information on sample collection.⁽¹⁾ Samples 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 sample should be inoculated onto an appropriate transport medium and refrigerated until inoculation.

Method of Use: Allow medium to warm to room temperature prior to inoculation. Consult references for information concerning inoculation procedures.⁽¹⁻³⁾

1. Spread a 0.1mL or 1.0mL sample over the surface of the medium and streak the sample to obtain isolated colonies. If using a swab, inoculate a small portion of the plate and streak the sample to obtain isolated colonies.
2. Incubate plates aerobically for 24 to 48 hours at 35-37°C and observe growth for characteristic hemolysis patterns and/or a greening or darkening of the medium surrounding colonies which indicates esculin hydrolysis.
3. Perform further testing as required by regulation or laboratory procedure for complete identification.

INTERPRETATION OF RESULTS

Observe colonies for alpha-, beta-, or non-hemolytic (gamma-) patterns. Examine colonies for a greening or darkening of the medium surrounding colonies, which is indicative of esculin hydrolysis. Consult listed references for appropriate interpretation of results or for further identification methods.⁽¹⁾

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.

Reactions given are typical of pure-isolates of the organism. Samples may contain no organism, one organism, or multiple organisms. Interpret results from well isolated colonies only to avoid erroneous or mixed results.

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, swabs, applicator sticks, other culture media, incinerators, 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>Enterococcus faecalis</i> ATCC® 29212	A	24-48hr	35°C	Aerobic	Growth; greening of media around colonies
<i>Staphylococcus aureus</i> ATCC® 25923	A	24hr	35°C	Aerobic	Growth; beta-hemolysis
<i>Escherichia coli</i> ATCC® 25922	A	24hr	35°C	Aerobic	Growth
<i>Streptococcus agalactiae</i> ATCC® 12386	A	24hr	35°C	Aerobic	Growth; beta-hemolysis

* 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

TSA (Tryptic Soy Agar) with Blood and 0.1% Esculin should appear opaque and cherry red in color.

REFERENCES

1. American Public Health Association. *Standard Methods for the Examination of Dairy Products*, APHA, Washington, D.C.

2. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods*, APHA, Washington, D.C.

3. U.S. Food and Drug Administration. *Bacteriological Analytical Manual*. Arlington, VA
<http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm>

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

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[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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