

# Instructions for Use

## CRITERION™ TRIPLE SUGAR IRON (TSI) AGAR

<a href="#">Cat. no. C7110</a>	CRITERION™ Triple Sugar Iron (TSI) Agar	130gm
<a href="#">Cat. no. C7111</a>	CRITERION™ Triple Sugar Iron (TSI) Agar	500gm
<a href="#">Cat. no. C7112</a>	CRITERION™ Triple Sugar Iron (TSI) Agar	2kg
<a href="#">Cat. no. C7113</a>	CRITERION™ Triple Sugar Iron (TSI) Agar	10kg
Cat. no. C7114	CRITERION™ Triple Sugar Iron (TSI) Agar	50kg

### INTENDED USE

Hardy Diagnostics CRITERION™ Triple Sugar Iron (TSI) Agar is recommended for use in the differentiation of Enterobacteriaceae by their ability to ferment glucose, lactose, and sucrose, and produce hydrogen sulfide.

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

In 1917 Sulkin and Willett described a medium containing the carbohydrates glucose, lactose, and sucrose, and iron salts.<sup>(8)</sup> The medium showed fermentation of these carbohydrates, as well as hydrogen sulfide production. Hajna modified the medium in 1945 to contain phenol red as the pH indicator, and is the formulation still in use today.<sup>(3)</sup>

Glucose is added to the medium since most enteric pathogens uniformly ferment this carbohydrate. Lactose and sucrose are added in ten times the amount of glucose, as most enteric pathogens do not ferment these sugars. As a result, non-pathogenic enterics which do ferment these sugars produce acid in the slant. Pathogenic enterics produce an initially acid slant from the low concentration of glucose, but as growth continues it changes to the alkaline reaction. Sodium thiosulfate is incorporated into the medium as a source of hydrogen sulfide. Ferrous ammonium sulfate serves as the indicator, which turns the butt black in the presence of free hydrogen sulfide gas. Enteric organisms that are capable of fermenting glucose will produce acid (a yellow butt and a red slant), a positive hydrogen sulfide result. Gas production may result and is seen as cracks and bubbles in the medium. If the slant and butt become alkaline, glucose has not been fermented. Organisms showing this reaction are defined as non-fermenters, and derive their nutrients from the peptones present in the medium.

TSI Agar is contained in a tube and is slanted to form a deep butt and short slant. Inoculation is performed with a straight needle by stabbing to the base of the butt, and streaking the slant when the needle is removed. The cap is replaced loosely to facilitate an aerobic atmosphere.

### FORMULA

Gram weight per liter:	65.0gm/L

Pancreatic Digest of Casein	15.0gm
Lactose	10.0gm
Sucrose	10.0gm
Sodium Chloride	5.0gm
Peptic Digest of Animal Tissue	5.0gm
Yeast Extract	3.0gm
Beef Extract	3.0gm
Dextrose	1.0gm
Ferric Ammonium Citrate	0.5gm
Sodium Thiosulfate	0.3gm
Phenol Red	0.024gm
Agar	12.0gm

Final pH 7.3 +/- 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 pink.

Store the prepared culture medium 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.

## METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Suspend 65.0gm of the dehydrated culture media in 1 liter of distilled or deionized water.
2. Heat to boiling and mix to dissolve completely.
3. Sterilize in the autoclave at 121°C. for 15 minutes.
4. Cool to 45-50°C. and aseptically add enrichments, if desired.

## 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. L50.

## 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.

It is important to stab the butt of the medium. Failure to stab the butt invalidates this test. The integrity of the agar must be maintained when stabbing. Caps must be loosened during this test or erroneous results will occur.

TSI Agar must be read within the 18-24 hour stated incubation period. A false-positive reaction may be observed if read too early. A false-negative reaction may be observed if read later than 24 hours.

An organism that produces hydrogen sulfide may mask acid production in the butt of the medium. However, hydrogen sulfide production requires an acid environment, thus the butt portion should be considered acid. TSI is not as sensitive in detecting hydrogen sulfide in comparison to other iron containing mediums, such as Sulfide Indole Motility (SIM) Medium. Thus, organisms that have weak hydrogen sulfide production may show only trace hydrogen sulfide activity, or none at all.

Certain species or strains may give delayed reactions or completely fail to ferment the carbohydrate in the stated manner. However, if the organism fails to ferment glucose within 48 hours, it most likely is not in the Enterobacteriaceae family.

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:

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Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Salmonella enterica</i> ATCC® 14028	C	18-24hr	35°C	Aerobic	Growth; red slant, yellow butt, gas positive, black butt (H <sub>2</sub> S produced)
<i>Escherichia coli</i> ATCC® 25922	C	18-24hr	35°C	Aerobic	Growth; yellow slant, yellow butt, gas positive, no H <sub>2</sub> S produced
<i>Pseudomonas aeruginosa</i> ATCC® 27853	C	18-24hr	35°C	Aerobic	Growth; red slant, red butt, no gas, no H <sub>2</sub> S produced

\* 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™ Triple Sugar Iron (TSI) Agar powder should appear homogeneous, free-flowing, and pink in color. The prepared media should appear slightly opalescent with a possible slight precipitate, 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. Hajna. 1945. *J. Bacteriol.*; 49:516.
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. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.
8. Sulkin and Willett. 1917. *J. Med. Research*; 37:225.

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



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[Ordering Information](#)

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