IFU

Instructions for Use

SELENITE CYSTINE BROTH

Cat. no. K69	Selenite Cystine Broth, 16x125mm Tube, 10ml	20 tubes/box

INTENDED USE

Hardy Diagnostics Selenite Cystine Broth is recommended for the selective enrichment of *Salmonella* spp. in clinical and non-clinical specimens.

SUMMARY

Leifson, in 1936, described the ability of Selenite Broth to enrich the cultivation of salmonellas while inhibiting other microorganisms. Leifson found coliforms and fecal streptococci to be inhibited by selenite, thereby permitting growth of *Salmonella* organisms.(7)

Selenite Cystine Broth is a modification of Leifson's formulation. The Food and Drug Administration first proposed the cystine formulation for use as an enrichment medium for detecting *Salmonella* in food materials. It is useful in detecting *Salmonella* when low numbers of organisms are present in stool.(2) It is also recommended for use in detecting *Salmonella* in food and water.(8-9)

Amino acids and other nitrogenous substances are provided by enzymatic digests of casein and animal tissue. L-Cystine is incorporated into the medium to improve the recovery of *Salmonella* . Phosphate is added to maintain a stable pH, in addition to decreasing the toxicity of selenite. Lactose also serves to maintain an optimal pH. Bacteria that reduce selenite produce alkali, which increases pH. Acid produced by lactose fermentation causes a decrease in pH, thereby maintaining a neutral or slightly decreased pH. Gram-positive organisms are inhibited by the presence of sodium selenite.

FORMULA

Ingredients per liter of deionized water:*

Sodium Phosphate	10.0gm
Tryptone	5.0gm
Lactose	4.0gm
Sodium Selenite	4.0gm
L-Cystine	0.01gm

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 2-8ºC. Products should not be used if there are any signs of deterioration, 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: Consult listed references for information on specimen collection.[1-4, 6] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. Specimens should be delivered to the laboratory within 2-3 hours. Special attention is required for stools. They should be collected early in the course of the disease and need to be cultured within two hours after collection. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport medium and refrigerated until inoculation.

Method of use:

1. Place 1.0gm of feces or 1ml of liquid stool in tube. Swab specimens may be inserted directly into the broth.

2. Emulsify the specimen thoroughly.

3. Incubate aerobically for 18 to 24 hours at 35ºC.

4. Place one to two drops of the incubated broth onto selective plate media, such as MacConkey or Hektoen Enteric (HE) Agar and streak for isolated colonies.

5. Incubate aerobically at 35ºC.

6. Examine for pathogens in 18-48 hours.

INTERPRETATION OF RESULTS

Culture analysis is made from the media to which the enriched specimen is subcultured. Consult listed references for the interpretation of growth and other identification tests to identify growth of organism in the medium to which subculture has been made.[1-6]

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.

The recovery of many *Salmonella* is greatly jeopardized if stool specimens remain unpreserved for more than three hours before processing. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport medium and refrigerated until inoculation.

A brick red precipitate may appear if Selenite Cystine Broth is overheated during preparation or exposed to excessive moisture during storage.

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	
Salmonella enterica ATCC® 14028	I	24hr	35°C	Aerobic	Growth
Shigella sonnei ATCC® 9290	I	24hr	35°C	Aerobic	Growth**
Escherichia coli ATCC® 25922	I	24hr	35°C	Aerobic	Growth

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

****Note:** Selenite Cystine Broth is inoculated with organism, incubated for 18-24 hours, then subcultured to a MacConkey Agar plate. The MacConkey plate should show heavy growth of *Salmonella* after 24 hours. *E. coli* will show partial to complete inhibition. *Shigella* should grow but recovery is variable.

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

Selenite Cystine Broth should appear clear, with a slight opalescence and very light precipitate, and light orange in color.

REFERENCES

1. Anderson, N.L., et al. 2005. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

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

6. *Quality Assurance for Commercially Prepared Microbiological Culture Media* , M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

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

8. Speck. 1984. *Compendium of Methods for the Microbiological Examination of Foods* , 2nd ed. APHA, Washington, D.C.

9. Greenberg, et al. 1985. *Standard Methods for the Examination of Water and Wastewater* , 16th ed. APHA, Washington, D.C.

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