IFU

Instructions for Use

TETRATHIONATE BROTH

Cat. no. K65	Tetrathionate Broth, 16x125mm Tube, 10ml	20 tubes/box
Cat. no. U165	Tetrathionate Broth, 1L PET Bottle, 1000ml	10 bottles/box
Cat. no. K164	Tetrathionate Broth with Brilliant Green, 16x125mm Tube, 10ml	20 tubes/box
Cat. no. Z129	Iodine-Iodide Solution, 2oz. Glass Bottle, 50ml**	50ml
Cat. no. Z139	Iodine-Iodide Solution, 1L Polycarbonate Bottle, 990ml**	990ml
** sold separately		

INTENDED USE

Hardy Diagnostics Tetrathionate Broth, with the addition of Iodine-Iodide Solution (Cat. no. Z129 or Z139), is recommended for the selective enrichment of *Salmonella* spp.

Hardy Diagnostics Tetrathionate Broth with Brilliant Green, along with the addition of Iodine-Iodide Solution (Cat. no. Z129 or Z139), is recommended for use as a selective media for the isolation of *Salmonella* spp. from pharmaceutical products, food, meat, and water.

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

SUMMARY

Mueller first described use of Tetrathionate Broth for the cultivation of *Salmonella* spp.[12] He found the medium to be inhibitory to lactose-fermenting *Enterobacteriaceae* while allowing unrestricted growth of *Salmonellae*. Schaeffer later demonstrated the medium to posses enrichment properties for *Salmonellae*.[13] Formula modifications were made by Kauffman who reported enhanced recovery of *Salmonella* spp.[14,15]

Tetrathionate Broth contains bile salts which are inhibitory to gram-positive and gram-negative microorganisms other than *Salmonella* spp. Iodine-Iodide Solution (Cat. no. Z129 or Z139) is added to the prepared medium just prior to inoculation. Addition of the Iodine-Iodide Solution promotes production of tetrathionate which inhibits the normal intestinal flora of fecal specimens.[5] The addition of brilliant green to the medium aids in the suppression of predominantly gram-positive bacteria.

Hardy Diagnostics Tetrathionate Broth conforms to the formulation recommended by the American Public Health Association (APHA), the Food and Drug Administration (FDA), and The United States Pharmacopeia (USP).[8-11]

FORMULA

Ingredients per liter of deionized water:*

Tetrathionate Broth (Cat. no. K65 and U165):

Sodium Thiosulfate	30.0gm
Calcium Carbonate	10.0gm
Pancreatic Digest of Casein	2.5gm
Peptic Digest of Animal Tissue	2.5gm
Bile Salts	1.0gm

In addition, Tetrathionate Broth with Brilliant Green (Cat. no. K164) contains:	
Brilliant Green	0.1gm

Final pH 8.4 +/- 0.2 at 25ºC.

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

Iodine-Iodide Solution (Cat. no. Z129 or Z139) not included:**	
Iodine	15.0gm
Potassium Iodide	12.5gm
Deionized Water	50.0ml

**** sold separately**

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-30ºC.(2-8ºC for Cat. no. K65 and K164). Products should not be used if there are any signs of contamination, deterioration, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing. Iodine-Iodide Solution (Cat. no. Z129 or Z139) should be stored in the dark.**

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

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. 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. Immediately before inoculation, add 0.2ml (approximately four drops) of Iodine-Iodide Solution (Cat. no. Z129 or Z139) to each 10ml tube of Tetrathionate Broth.

2. Place 1.0 to 3.0gm (heavy inoculum) of specimen to each tube. Swab specimens may be inserted directly into the broth.

3. Emulsify the specimen thoroughly.

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

5. Place one to two drops of the incubated broth onto selective plate media, such as MacConkey Agar (Cat. no. G35) and streak for isolated colonies.

6. Incubate aerobically at 35ºC.

7. Examine for presence of typical colonies at 18-48 hours.

Industrial Samples (e.g. food, potable water):
Refer to listed references for the isolation and identification of *Salmonella* spp. from non-clinical samples.[10,11,16]

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 biochemical and serological tests necessary to identify growth of organism in the medium to which subculture has been made.[1-4,6,10,11,16]

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.

Due to the nutritional requirements and inhibitory characteristics of the microorganisms obtained, microbes other than salmonellae, such as *Morganella morganii* and some Enterobacteriaceae, may grow in this medium.

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, slides, staining supplies, Iodine-Iodide Solution (Cat. no. Z129 or Z139), other culture media, microscopes, 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	18-24hr	35°C	Aerobic	Growth on MacConkey upon subculture
*Escherichia coli*** ATCC® 25922	I	18-24hr	35°C	Aerobic	Partial to complete inhibition on MacConkey upon subculture
Tetrathionate Broth with Brilliant Green (Cat. no. K164):					
Enterococcus faecalis ATCC® 29212	I	18-24 hrs	35°C	Aerobic	Inhibited upon subculture to MacConkey
*Salmonella enterica*** ATCC® 14028	I	18-24hr	35°C	Aerobic	Growth on MacConkey upon subculture
*Escherichia coli*** ATCC® 25922	I	18-24hr	35°C	Aerobic	Partial to complete inhibition on MacConkey upon subculture

Note: Tetrathionate Broth is inoculated with organism, incubated for 18-24 hours, then subcultured to a MacConkey Agar plate.

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

** Recommended strains for User Quality Control.

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

- Tetrathionate Broth should appear milky white and clear, with a precipitate, upon standing.
- Tetrathionate Broth with Brilliant Green should appear opaque, light green with no precipitate or debris.
- Iodine-Iodide Solution should appear dark amber to brown in color.**
  ** Iodine-Iodide Solution is sold separately.

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. Jorgensen., et al. *Manual of Clinical Microbiology*, 9th ed. 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. *The United States Pharmacopeia*, 21st rev. 1985. U.S. Pharmacopeial Convention, Rockville, MD.

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

10. Greenberg, et al. 1992. *Standard Methods for the Examination of Water and Wastewater*, 18th ed. APHA, Washington, D.C.

11. U.S. Food and Drug Administration. *Bacteriological Analytical Manual*. AOAC, Arlington, VA. www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm.

12. *Compt. Rend. Soc. Biol.*; 89:434, 1923.

13. *Zentr. Bakt. I. Abt. Orig.*; 133:458, 1935.

14. *Zentr. Bakt. I. Abt. Orig.*; 119:148, 1930.

15. *Zeit, Hyg.*; 117:26, 1935.

16. *Official Methods of Analysis*, 15th ed. 1990. AOAC, Arlington, VA.

IFU-10789[C]

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:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

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

HDQA 2207D [D]