IFU

Instructions for Use

CRITERION™ RAPPAPORT-VASSILIADIS BROTH

Cat. no. C8780	CRITERION™ Rappaport-Vassiliadis Broth	54.8gm
Cat. no. C8781	CRITERION™ Rappaport-Vassiliadis Broth	500gm
Cat. no. C8782	CRITERION™ Rappaport-Vassiliadis Broth	2kg
Cat. no. C8783	CRITERION™ Rappaport-Vassiliadis Broth	10kg
Cat. no. C8784	CRITERION™ Rappaport-Vassiliadis Broth	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Rappaport-Vassiliadis Broth is recommended for the selective enrichment of *Salmonella* spp. from food samples and conforms to the Harmonized USP/EP/JP requirements.

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

Rappaport Medium was initially developed by Rappaport et al. in 1956 as an alternative to Tetrathionate Broth for the enrichment of *Salmonella*.[1] This formulation features magnesium chloride to inhibit *Proteus* spp. and *Escherichia coli*; malachite green to inhibit coliforms, and a high osmotic pressure and/or low pH to inhibit accompanying microbial flora other than *Salmonella*. In 1976, Vassiliadis et al. described a modification of Rappaport Medium called R10.[2] This formula features a reduced concentration of malachite green and an increased incubation temperature. It was later shown in 1989 by Peterz et al. that incubation at 41.5 +/-0.5ºC. for 24 hours significantly improved the recovery of *Salmonella* spp.[3]

CRITERION™ Rappaport-Vassiliadis Broth is a modification of Rappaport-Vassiliadis R10 Broth and uses soy peptone as the nitrogen and vitamin source. Studies show that soy peptone enhances the growth of *Salmonella* spp. and counteracts the risk of potential Bovine Spongiform Encephalopathy (BSE) exposure associated with bovine derived products. CRITERION™ Rappaport-Vassiliadis Broth conforms to the Harmonized United States Pharmacopoeia (USP), European Pharmacopoeia (EU), and Japanese Pharmacopoeia (JP).[4-7] The medium selectively enriches for *Salmonella* spp., although malachite green may inhibit the growth of more sensitive strains of *Salmonella*, such as *S. typhi* and *S. choleraesuis*.

FORMULA*

Gram weight per liter:	27.4gm/L
Magnesium Chloride, Anhydrous	13.4gm
Sodium Chloride	8.0gm

Soy Peptone	4.5gm
Dipotassium Phosphate	1.26gm
Monopotassium Phosphate	0.18gm
Malachite Green	0.036gm

Final pH 5.2 +/- 0.2 at 25ºC.

* Adjusted and/or supplemented to meet performance criteria.

NOTE: Since magnesium chloride hexahydrate contains too much water to be effectively used in the manufacture of dehydrated culture media, magnesium chloride anhydrous is used as an acceptable alternative. The actual overall concentration of magnesium chloride is equivalent with this substitution; yet, the adjustment requires additional modifications to the USP formulation to correct for molecular weight. None of these modifications affect the overall performance or intended use of this item as indicated on the Certificate of Analysis, which shows harmonized growth promotion criteria are met per USP.[4-7]

STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-30ºC. 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 pale green.

Store the prepared culture media at 2-8ºC.

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 27.4gm of the dehydrated culture media in one liter of distilled or deionized water. Stir to mix thoroughly.

2. Heat as necessary to dissolve completely.

3. Dispense 10ml into glass tubes and cap loosely.

4. Sterilize in the autoclave using a validated cycle, at a temperature not exceeding 115ºC.

PROCEDURE AND INTERPRETATION OF RESULTS

For information on specific procedures and the interpretation of results, consult appropriate reference guidelines or refer to the prepared media Instructions for Use (IFU) for Cat. No. K246.

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.

Certain strains of *Salmonella*, such as *S. typhi* and *S. choleraesuis*, may be inhibited on this medium. Therefore, isolation techniques should include a variety of enrichment broths and selective media for best recovery.

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:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Salmonella enterica ATCC® 14028	J	18-24hr	35°C	Aerobic	Positive upon subculture to XLD
Staphylococcus aureus ATCC® 6538	B	48hr	35°C	Aerobic	Inhibited upon subculture to XLD

* 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™ Rappaport-Vassiliadis Broth powder should appear homogeneous, free-flowing, and pale green in color. The prepared media should appear clear and blue in color.

REFERENCES

1. Rappaport, F., N. Konforti, and B. Navon. 1956. A New Enrichment Medium for Certain Salmonellae. *J. Clin. Pathol.*; 9:261-266.

2. Vassiliadis, P., D. Trichoppoulos, A. Kalandidi, and E. Xirouchaki. 1978. Isolation of Salmonellae from Sewage with a New Procedure of Enrichment. *J. Appl. Bacteriol.*; 44:233-239.

3. Peterz, M. C. Wiberg, and P. Norberg. 1989. The Effect of Incubation Temperature and Magnesium Chloride Concentration on Growth of *Salmonella* in Homemade and Commercially Available Dehydrated Rappaport-Vassiliadis Broths. *J. Appl. Bacteriol.*; 66:523-528.

4.*United States Pharmacopoeia and National Formulary* (USP-NF). Rockville, MD: United States Pharmacopeial Convention.

5. Directorate for the Quality of Medicines of the Council of Europe (EDQM). 2007. The European Pharmacopoeia, Amended Chapters 2.6.12, 2.6.13, 5.1.4, Council of Europe, 67075 Strasbourg Cedex, France.

6. Japanese Pharmacopoeia. 2007. Society of Japanese Pharmacopoeia. Amended Chapters 35.1, 35.2, 7. The Minister of Health, Labor, and Welfare.

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

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

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

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

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

12. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater,* APHA, Washington, D.C.

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

14. Association of Official Analytical Chemists. *Official Methods of Analysis*^{sm}, AOAC, Washington, D.C.

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

HDQA 2207B [D]