IFU

Instructions for Use

ARYLSULFATASE BROTH

Cat. no. K93	Arylsulfatase Broth 0.001M, 16x125mm Tube, 2ml	20 tubes/box
Cat. no. K94	Arylsulfatase Broth 0.003M, 16x125mm Tube, 2ml	20 tubes/box

INTENDED USE

Hardy Diagnostics Arylsulfatase Broths are chemically-defined media used in the differentiation of pathogenic mycobacteria species based on their ability to produce arylsulfatase.

The 0.001M Arylsulfatase Broth is used in a 3-day test to detect arylsulfatase activity in rapidly-growing mycobacteria species. The 0.003M Arylsulfatase Broth is used in a two-week test for the detection of arylsulfatase in slow-growing mycobacteria species.

SUMMARY

Arylsulfatase is produced by many mycobacterial species in varying concentrations.[1-3] The ability to produce a detectable level of arylsulfatase is a biochemical characteristic used in the differentiation of some *Mycobacterium* species.[4-8]

The test is performed by inoculating a broth containing tripotassium phenolphthalein disulfate with a *Mycobacterium* isolate.[7] If arylsulfatase is produced, it splits the phenolphthalein substrate, releasing free phenolphthalein, which turns pink to red when alkali is added to the medium.

The 0.001M Broth is used for rapidly-growing arylsulfatase producers, such as *Mycobacterium fortuitum* and *Mycobacterium chelonae*.

The 0.003M Broth is used for slow-growing mycobacteria such as *Mycobacterium szulgai*, *Mycobacterium trivale*, *Mycobacterium xenopi*, *Mycobacterium tuberculosis*, and *Mycobacterium flavescens*.[4,7]

FORMULA

Ingredients per 900ml of deionized water:*

Disodium Phosphate	2.5gm
Monopotassium Phosphate	1.0gm
Ammonium Sulfate	0.5gm
Monosodium Glutamate	0.5gm
Sodium Citrate	0.4gm
Magnesium Sulfate	0.05gm

Ferric Ammonium Citrate	0.04gm
Pyridoxine	1.0mg
Zinc Sulfate	1.0mg
Copper Sulfate	1.0mg
Biotin	0.5mg
Calcium Chloride	0.5mg
ADC Enrichment	100.0ml
Tripotassium Phenolphthalein Disulfate**	

** Arylsulfatase Broth (0.001M) contains 0.65gm/L Tripotassium Phenolphthalein Disulfate.

** Arylsulfatase Broth (0.003M) contains 1.95gm/L Tripotassium Phenolphthalein Disulfate.

Final pH 6.6 +/- 0.3 at 25ºC.

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

ADC Enrichment	
Ingredients per liter of deionized water:*	
Bovine Albumin, Fraction V	50.0gm
Dextrose	20.0gm
Sodium Chloride	8.5gm
Catalase	0.03gm

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. 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 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: These media are not suitable for use directly with clinical specimens or other sources containing mixed microbial flora. Consult appropriate references for more information.[4-8]

Organisms to be cultivated must first be isolated in pure culture on appropriate medium.

Inoculate the broth with 0.1ml of a 7-day Middlebrook 7H-9 liquid culture (Cat. no. C32) or heavily inoculate with organisms cultured on a solid medium.

Incubate the tubes at 35ºC. with tightened caps in an aerobic atmosphere without added CO_2. Remove the 0.001M Broth after three days and add no more than six drops of 1M sodium carbonate solution (10.6gm anhydrous Na_2 CO_3 in 100ml of water), and observe for a color change.

Incubate the 0.003M Broth for two weeks, then remove and add six drops of the 1M sodium carbonate solution.

INTERPRETATION OF RESULTS

A change in the color of the medium to pink or red following the addition of sodium carbonate is a positive reaction. The medium remains colorless if negative. Determine the intensity of the color reaction and record as follows:

Color reaction	**Score**
No color change	(-)
Pale Pink	(1+)
Pink	(2+)
Light Red	(3+)
Red	(4+)
Deep Red	(5+)

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.

For identification, the organism must be in pure culture. Morphological, biochemical and /or serological tests should be performed for final identification. Consult appropriate references for detailed information and recommended procedures.[7,8]

Always test a few tubes of the prepared, uninoculated substrate medium for free phenolphthalein by adding a few drops of 2N Sodium Carbonate. Free phenolphthalein in the media can lead to false-positive 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, 1M sodium carbonate solution, 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	Arylsulfatase Broth	Reaction
Mycobacterium fortuitum ATCC® 6841	0.001M	Pale pink to pink color
Mycobacterium phlei ATCC® 11758	0.001M	No color reaction
Mycobacterium xenopi ATCC® 19250	0.003M	Pink to red color
Mycobacterium tuberculosis ATCC® 25177	0.003M	No color reaction

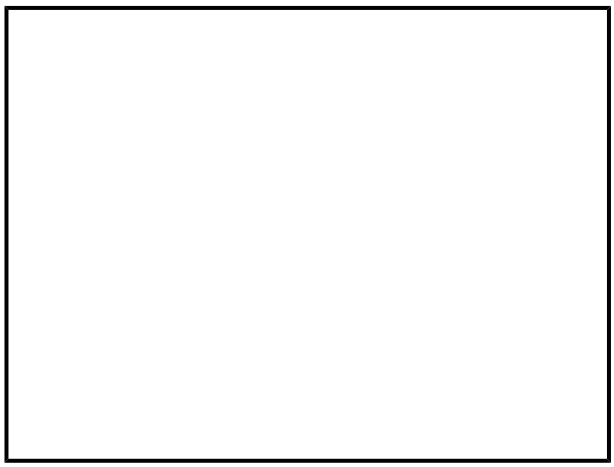
* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

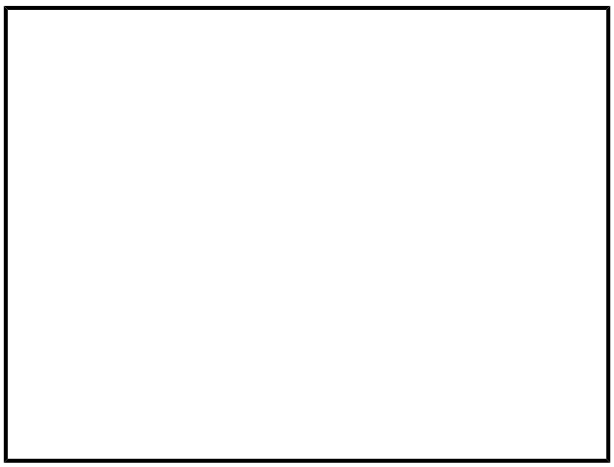
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory 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

Arylsulfatase Broth should appear clear and colorless, with no precipitate.



Mycobacterium fortuitum (ATCC® 6841) growing in 0.001M Arylsulfatase Broth (Cat. no. K93). Incubated aerobically for three days at 35ºC. Subsequent to incubation, six drops of 1M sodium carbonate solution (Cat. no. Z102) were added to the tube. The pink color change was indicative as positive for the production of arylsulfatase.



Mycobacterium tuberculosis(ATCC® 25177) growing in 0.001M Arylsulfatase Broth (Cat. no. K93). Incubated aerobically for three days at 35ºC. Subsequent to incubation, six drops of 1M sodium carbonate solution (Cat. no. Z102) were added to the tube. No pink color change was indicative as negative for the production of arylsulfatase.

REFERENCES

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3. Whitehead, J.E.M., et al. Arylsulfatase Activity of Mycobacteria. *J. Path. Bact.*; 65:451-460.

4. 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.

5. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

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7. Vestal, A.L. 1975. " *Procedures for the Isolation and Identification of Mycobacteria*", DHEW Publication No. (CDC) 75-8230. U.S. Department of Health, Education, and Welfare. Center for Disease Control, Atlanta, GA.

8. Sommers, H.M., and J.P. Russell. 1967. *Clinically Significant Mycobacteria: Their Recognition and Identification.* American Society of Clinical Pathologists, Chicago, IL.

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

HDQA 2207F [D]