IFU

Instructions for Use

# CRITERION™ AMPICILLIN DEXTRIN AGAR BASE

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|---|---|---|
| Cat. no. C7580 | CRITERION™ Ampicillin Dextrin Agar Base | 90gm |
| Cat. no. C7581 | CRITERION™ Ampicillin Dextrin Agar Base | 500gm |
| Cat. no. C7582 | CRITERION™ Ampicillin Dextrin Agar Base | 2kg |
| Cat. no. C7583 | CRITERION™ Ampicillin Dextrin Agar Base | 10kg |
| Cat. no. C7584 | CRITERION™ Ampicillin Dextrin Agar Base | 50kg |

## INTENDED USE

Hardy Diagnostics CRITERION™ Ampicillin Dextrin Agar Base is recommended for the selective isolation, differentiation, and quantitative enumeration of *Aeromonas* species from water, food and environmental samples by the membrane filter method.

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

*Aeromonas* spp. are gram-negative, oxidase-positive, non-spore-forming, rod-shaped, motile, facultatively anaerobic bacteria that are autochthonous to fresh and marine water settings and to some aquatic fauna.[1] In addition, they are readily isolated from both nutrient-rich and nutrient-poor environments and their numbers vary depending upon the temperature and trophic state of the territory.[2,5] Rising pollution levels and nutrient loading due to runoff may yield substantially greater and greater populations of these microbes, and may also affect the distribution of microorganisms in a given region. However, aeromonads have been isolated from municipal drinking-water systems, sometimes at high levels; consequently, there is great potential for them to enter distribution systems if water treatment is ineffective.[1,2,5]

Recent medical reports have increasingly associated *Aeromonas* spp. with human infections.[2] Mesophilic aeromonads have been isolated from patients with gastroenteritis, though their etiology in disease is unclear. They are associated with sepsis and wounds, and with eye, respiratory tract, and other systemic infections, particularly in young children and immunocompromised patients. Moreover, many systemic infections are the result of contamination of lacerations and fractures from *Aeromonas*-rich waters, and the severity of disease appears to correlate strongly with enterotoxin or toxigenic factor-producing species.[1]

Due to the increasing medical significance of these microorganisms, Havelaar et. al. designed a culture media based on a modification of the positive aspects of mA-Agar, described by Rippey and Cabelli, and Dextrin-Fuchsin-Sulphite (DFS) Agar to create Ampicillin Dextrin Agar.[3,4] Hardy Diagnostics' CRITERION™ Ampicillin Dextrin Agar Base, when combined with antimicrobial agents such as Ampicillin (Cat. no. SR136E), and vancomycin or the vibriostatic agent O129, is a selective medium that partially inhibits the growth of non-target bacterial species while allowing most *Aeromonas* spp. to grow.[5] Consequently, *Aeromonas* growth on this medium can be presumptively identified by the fermentation of dextrin and the presence of yellow colonies. Yellow colonies can then be counted and confirmed by

testing for the presence of cytochrome c (oxidase test), the ability to ferment trehalose, and the production of indole.[1,2,5]

## FORMULA*

| Gram weight per liter: | 36.7gm/L |
|---|---|
| | |
| Agar | 15.0gm |
| Dextrin | 10.0gm |
| Tryptose | 5.0gm |
| Sodium Chloride | 3.0gm |
| Yeast Extract | 2.0gm |
| Potassium Chloride | 2.0gm |
| Magnesium Sulphate Heptahydrate | 0.2gm |
| Ferric Chloride | 0.1gm |
| Sodium Deoxycholate | 0.1gm |
| Bromothymol Blue | 0.08gm |

Final pH 8.0 +/- 0.2 at 25ºC.

Note: The addition of 10mg of sterile Ampicillin (Cat. no. SR136E) is needed to complete the ADA formula. An additional 2mg of sterile vancomycin may also be added to make Ampicillin Dextrin Agar with Vancomycin (ADA-V). If testing for *Aeromonas* in sea water, 50mg of the vibriostatic agent O129 may be added to inhibit the growth of *Vibrio* spp.

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

## STORAGE AND SHELF LIFE

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

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 36.7gm of the dehydrated culture media in 1 liter of distilled or deionized water. Stir to mix thoroughly.

2. Heat to boiling to dissolve completely.

3. Sterilize in the autoclave at 121ºC. for 15 minutes.

4. Cool to 45-50ºC.

5. Add 10mg of sterile Ampicillin (Cat. no. SR136E) to inhibit *Enterococcus faecalis* .

Note: Alternatively, an additional 2mg of sterile vancomycin may be added to create Ampicillin Dextrin Agar with Vancomycin (ADA-V) to enumerate *Aeromonas* in finished waters. If detecting *Aeromonas* spp. in sea water, 50mg of the vibriostatic agent O129 can be added to the ADA medium.

6. Aseptically pour appropriate volume into desired sterile containers.

## 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. G180.

## 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.

In some instances, *Enterococcus* spp. are reported to produce pinpoint-sized yellow colonies on Ampicillin Dextrose Agar.[5] Confirmation of presumptive *Aeromonas* colonies is necessary to rule out false-positives.

When examining food and environmental samples, where competing flora may be dominated by *Pseudomonas* spp., incubation at 37ºC. for 24 hours yields the best results due to the inhibition of most *Pseudomonas* spp. as proposed by Rippey and Cabelli.[2] At lower temperatures, the growth of *P. aeruginosa* may overwhelm the sample, making quantitative recovery extremely difficult.

Incubation under anaerobic conditions results in a lower rate of dextrin fermentation and faintly yellow to yellowish-green *Aeromonas* colonies that may fade to green upon removal of cultures from the incubator.[4]

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, Ampicillin (Cat. no. SR136E), Vancomycin, O129, membrane filters, 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 | |
| *Aeromonas hydrophila* ATCC® 35654 | A | 24-48hr | 15-30°C | Aerobic | Growth; yellow colonies |
| *Escherichia coli* ATCC® 25922 | A | 24-48hr | 15-30°C | Aerobic | Variable, if present yellow colonies |
| *Enterococcus faecalis* ATCC® 29212 | B | 24-48hr | 15-30°C | Aerobic | Partial to complete inhibition |

* 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™ Ampicillin Dextrin Agar Base powder should appear homogeneous, free-flowing, and beige in color. The prepared media should appear slightly opalescent, and blue in color.

## REFERENCES


1. Sartory et. al. 2006. *Guidelines for Drinking-Water Quality*. 3rd ed. WHO, Switzerland; 1-17.

2. Rippey and Cabelli. 1979. Membrane Filter Procedure for Enumeration of *Aeromonas hydrophila* in Fresh Waters. *Appl. and Environ. Micro.*; 38:108-113.

3. Corry, Curtis and Baird. 1995. *Culture Media for Food Microbiology*. Elsevier. New York, NY.

4. Havelaar, A.H., M.During and J.F.M.Versteegh. 1987. Ampicillin-dextrin agar medium for the enumeration of *Aeromonas* species in water by membrane filtration. *Society for Applied Bacteriology*.

5. Feige, M.A. 2001. Method 1605: *Aeromonas* in Finished Water by Membrane Filtration using Ampicillin-Dextrin Agar with Vancomycin (ADA-V). EPA. Washington, D.C.

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

7. Association of Official Analytical Chemists. *Official Methods of Analysis*[sm], AOAC, Washington, D.C.

8. Committee of Revision for The United States Pharmacopeia. 2004. *The United States Pharmacopeia*, 27th rev. United States Pharmacopeial Convention, Rockville, MD.

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

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

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



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



HDQA 2207B [D]