IFU

Instructions for Use

CRITERION™ R2A AGAR

Cat. no. C6720	CRITERION™ R2A Agar	30.4gm
Cat. no. C6721	CRITERION™ R2A Agar	500gm
Cat. no. C6722	CRITERION™ R2A Agar	2kg
Cat. no. C6723	CRITERION™ R2A Agar	10kg
Cat. no. C6724	CRITERION™ R2A Agar	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ R2A Agar is recommended for use in the pour plate and spread plate methods for enumeration of heterotrophic bacteria in water, especially potable water.

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

Reasoner and Geldreich, of the U.S. Environmental Protection Agency, developed R2A Agar for the recovery and isolation of aerobic and facultative anaerobic heterotrophic bacteria from treated potable water.[1,2]

R2A Agar, as compared to other media recommended for the heterotrophic plate count (HPC), contains reduced levels of peptone, yeast extract, and dextrose. The decreased nutrient level, along with the addition of sodium pyruvate, enhances the recovery of many stressed and chlorine-tolerant bacteria that are present in treated waters.[2] Also, the heterotrophic bacteria recovery method using R2A Agar requires incubation temperatures below routine laboratory requirements, which further enhances the recovery of many bacteria.[4,5]

This formula contains peptones that provide nitrogen, vitamins, amino acids, and minerals. Dextrose acts as a carbon source, while yeast extract is added for trace elements and vitamins. For the recovery of injured cells, soluble starch and sodium pyruvate are used to neutralize toxins. Magnesium sulfate provides magnesium cations and sulfate. Potassium phosphate buffers the pH of the media and agar is used for solidification.

FORMULA*

Gram weight per liter:	15.2gm/L
Casein Acid Hydrolysate	0.5gm
Yeast Extract	0.5gm
Dextrose	0.5gm

Soluble Starch	0.5gm
Dipotassium Phosphate	0.3gm
Sodium Pyruvate	0.3gm
Casein Peptone	0.25gm
Peptic Digest of Animal Tissue	0.25gm
Magnesium Sulfate	0.05gm
Agar	15.0gm

Final pH 7.2 +/- 0.2 at 25ºC.

* 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 light beige.

Store the prepared plated culture media at 2-8ºC.
Store the prepared tubed and bottled culture media at 2-30ºC.

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 15.2gm of the dehydrated culture media in 1 liter of distilled or deionized water. Stir to mix thoroughly.

2. Heat to boiling to dissolve completely. Do not overheat.

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

4. Cool to 45-50ºC. and dispense.

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. G54.

LIMITATIONS

The pour plate method is not highly recommended because the recovery of injured bacteria may be lessened by the heat of the media at 45ºC. For small sample volumes, the spread plate technique is most effective. When larger amounts of water need to be tested, the membrane filter method is suggested.

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.

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, petri dishes, membrane filters, 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	
Escherichia coli ATCC® 8739	J	1-3 days	35°C	Aerobic	Growth
Enterococcus faecalis ATCC® 29212	J	1-3 days	35°C	Aerobic	Growth
Staphylococcus aureus ATCC® 6538	J	1-3 days	35°C	Aerobic	Growth
Pseudomonas paraeruginosa ATCC® 9027	J	1-3 days	35°C	Aerobic	Growth
Bacillus spizizenii ATCC® 6633	J	1-3 days	35°C	Aerobic	Growth

* 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™ R2A Agar powder should appear homogeneous, free-flowing, and light beige in color. The prepared media should appear clear, slightly opalescent, and light amber in color.

REFERENCES

1. Reasoner, D.J., and Geldreich, E.E. 1979. Paper No. N7, Annual Meeting of The American Society for Microbiology.

2. Reasoner, D.J., and Geldreich, E.E. 1985. *Applied and Environmental Microbiology*; 49:1-7.

3. *Standard Methods for the Examination of Water and Wastewater*. 1995. 19th ed. American Public Health Association, Washington, D.C.

4. Stark and McCoy. 1938. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg.*, Abt. 2; 98:201.

5. Collins and Willoughby. 1962. *Arch. Mikrobiol.*; 43:294.

IFU-10240[B]

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 2207B [D]