

# Instructions for Use

## R2A AGAR

<a href="#">Cat. no. W390</a>	R2A Agar, SterEM™, Irradiated, Triple Bagged, 15x100mm Plate, 26ml	10 plates/bag
-------------------------------	--	---------------

## INTENDED USE

Hardy Diagnostics R2A Agar, Irradiated is recommended for the cultivation and enumeration of heterotrophic bacteria in water, especially potable water, and for use in testing high purity waters for pharmaceutical purposes.

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

## SUMMARY

R2A Agar is mentioned in the United States Pharmacopoeia, USP <1231> *Water for Pharmaceutical Purposes* as a suitable low-nutrient medium for optimal recovery of microorganisms from oligotrophic environments. USP <1231> also states it is essential to use a medium, such as R2A Agar, that has been demonstrated through validation studies as optimal for the microbiome in a particular water system.

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.<sup>(1,2)</sup>

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 present in treated waters.<sup>(2)</sup> Also, the heterotrophic bacteria recovery method using R2A Agar requires incubation temperatures below routine laboratory requirements, which further enhances the recovery of many stressed bacteria.<sup>(4,5)</sup>

The plates are triple bagged and sterilized by irradiation to promote a higher sterility assurance level.

## FORMULA

Ingredients per liter of deionized water:\*

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 25°C.

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

## STORAGE AND SHELF LIFE

Storage: Store at 15-30°C., and store media away from direct light. Media should not be used if there are any signs of contamination, deterioration, discoloration, or if the expiration date has passed. Product is light and temperature sensitive. Protect from light, excessive heat, moisture, and freezing.

Small amounts of condensation may be observed upon receipt, especially during seasonal (summer, winter, heat waves, etc.) weather shipments. If present, condensation should improve over time when the media is stored long-term under the labeled storage conditions.

**Inspect the packaging seals prior to opening and using the product. Do not use irradiated media if there is any damage to the packaging or breach of seals prior to use.**

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

**Before Use of Plates:** Due to the triple packaging, it is possible that variations in temperature and pressure during shipping and storage may cause condensation within the innermost sleeve surrounding the plates. If condensation of the packaging or plates is observed just prior to use, remove the plates from the innermost packaging in a controlled (e.g. ISO 5) environment and allow them to dry for 10-15 minutes before use.

The medium should be room temperature and the surface dry prior to use. To reduce the potential for cross-

contamination, it is strongly suggested that appropriate gowning and glove procedures, designated aseptic processing areas, appropriate sporicidal disinfectants, and environmental monitoring procedures be strictly followed to reduce the likelihood of accidental contamination.<sup>(1)</sup>

**Sample Collection:** Consult listed references for information on the collection of water samples.<sup>(3,6)</sup>

**Method of Use:** Refer to listed references for standard methods employed in membrane filter procedures.<sup>(3,6)</sup> Incubate media using appropriate atmospheric, temperature, and duration conditions as outlined by the test method. Count the number of colonies and report as the number of colony forming units (CFU).

### **Membrane Filter Method:**

*For more detailed instructions on sampling and membrane filtration procedures, consult Standard Methods for the Examination of Water and Wastewater.<sup>(3)</sup>*

1. Collect and prepare water samples in accordance with recommended guidelines.
2. After the sample has been filtered, aseptically remove the membrane filter from the filter base and roll it onto R2A Agar. **Avoid the formation of bubbles between the membrane and the agar surface.**
3. Invert the inoculated plate and incubate.
4. The recommended incubation for R2A Agar is 35°C. for at least 72 hours and up to 7 days. The medium can also be incubated at 20 or 28°C. for not less than 5 days, preferably up to 7 days.
5. After the incubation period, using an illuminated lens with 2-5x magnification, count and record the number of colonies.

### **Spread Plate Method:**

1. Prepare decimal dilutions in sterile diluent to obtain 30-300 CFU per plate.
2. Aseptically inoculate agar surface with 0.1ml of well mixed diluted sample.
3. Using a sterile spreader device, distribute the inoculum evenly over the entire agar surface.
4. The recommended incubation for R2A Agar is 35°C. for at least 72 hours and up to 7 days. The medium can also be incubated at 20 or 28°C. for not less than 5 days, preferably up to 7 days.

## **INTERPRETATION OF RESULTS**

The number of colonies present on the agar medium are reported as colony-forming units (CFU) per volume of sample. Also reported are: the test method used, incubation temperature and time, and R2A Agar (the test medium used).

The heterotrophic plate count is computed by dividing the total number of colonies or average number, in the case of duplicate plates, by the sample volume.

Consult listed references for standard methods in computing and reporting CFU counts.<sup>(3,6)</sup>

## **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.

Avoid repeated and/or extreme variations in temperatures during storage, as this can cause the release of excessive moisture from the media in the bags and plates.

If removing the outer and middle packaging and storing plates in the innermost sleeve long-term in a controlled

environment, 2-8°C storage conditions are recommended to prevent dehydration of the medium prior to use.

Storage at 2-8°C in the triple packaging may result in moisture build-up inside sealed packs.

Each sleeve of media includes a label with an irradiation indicator strip that turns from yellow to orange upon irradiation exposure (either the entire strip or just a portion). Inspect strips for evidence of a color change, which indicates successful irradiation. **Note:** the color intensity of indicator strips may fade or change if the label is exposed to ambient light during long-term storage. Protect from light and examine for color change immediately upon exposure to ambient lighting

The method of packaging disinfection using vaporized hydrogen peroxide (VHP) treatment has not been validated. Due to the use of breathable packaging materials, as well as folds and creases present on the packaging, users should consider validation of a non-vapor or alternative disinfection method.

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.

Consult appropriate regulatory agency for user QC requirements.

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, other culture media, [swabs](#), [impact air samplers](#), [sterile sample bags \(Cat. no. BAS381BX\)](#), 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*	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Escherichia coli</i> ATCC® 8739	J	1-3 days	35°C	Aerobic	Growth
<i>Enterococcus faecalis</i> ATCC® 29212	J	1-3 days	35°C	Aerobic	Growth
<i>Staphylococcus aureus</i> ATCC® 6538	J	1-3 days	35°C	Aerobic	Growth
<i>Pseudomonas paraeruginosa</i> ATCC® 9027	J	1-3 days	35°C	Aerobic	Growth
<i>Pseudomonas aeruginosa</i> ATCC® 27853	J	1-3 days	35°C	Aerobic	Growth
<i>Bacillus spizizenii</i> ATCC® 6633	J	1-3 days	35°C	Aerobic	Growth

Representative samples from each lot of irradiated media are held for seven days to confirm the media meet the validated sterilization process sterility assurance level (SAL) of 10<sup>-6</sup> following ANSI/AAMI/ISO 11137.

\* 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

Irradiated R2A Agar should appear clear, slightly opalescent, and light amber in color.



*Escherichia coli* (ATCC® 8739) filtered through a black membrane and growing on R2A Agar. Incubated aerobically for 24 hours at 35°C.

## 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. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*, APHA, 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.
6. The Official Compendia of Standards. *USP-NF*. United States Pharmacopeial Convention. Rockville, MD.

ATCC is a registered trademark of the American Type Culture Collection.



1430 West McCoy Lane, Santa Maria, CA 93455, USA

Phone: (805) 346-2766 ext. 5658

Fax: (805) 346-2760

Website: [HardyDiagnostics.com](http://HardyDiagnostics.com)

Email: [TechnicalServices@HardyDiagnostics.com](mailto: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.

Copyright© 2020 by Hardy Diagnostics. All rights reserved.

HDQA 2207D [D]