IFU

Instructions for Use

R2A AGAR

Cat. no. G03	R2A Agar, 15x60mm Plate, 11ml	10 plates/bag
Cat. no. G54	R2A Agar, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. G364	R2A Agar, without plate label, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. Q77	R2A Agar, 20x125mm Tube, 18ml	20 tubes/box
Cat. no. U354	R2A Agar, 16oz. Glass Bottle, 400ml	12 bottles/box
Cat. no. W530	R2A Agar, SterEM™, irradiated, triple bagged, 15x100mm plate, 26ml*	10 plates/bag
* A fourth sterile sample bag is included for packaging after the sample is collected.		

INTENDED USE

Hardy Diagnostics R2A Agar is recommended for enumeration of heterotrophic bacteria in water, especially potable water.

R2A Agar is mentioned in the United States Pharmacopoeia, USP <1231> *Water for Pharmaceutical Purposes* for use in testing hight-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.[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 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 stressed bacteria.[4,5]

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: Upon receipt, store plates at 2-8°C., store tubes and bottled media at 2-30°C, and store irradiated plates at 15-30°C. Products should not be used if there are any signs of contamination, deterioration (shrinking, cracking, or discoloration), or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

Do not use irradiated media if there is any damage to the packaging prior to use.

For irradiated media: Inspect each bag prior to opening and using the product.

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: For irradiated media, it is possible variation in temperature and pressure during shipping and storage may cause condensation on the innermost bag surrounding the plates. If condensation of the packaging or plates is observed, remove the plates from the innermost packaging in a sterile environment and allow them to dry for 10-15 minutes before use.

Sample Collection: Consult listed references for information on the collection of water samples.[3,6]

Method of Use: Allow medium to warm to room temperature prior to inoculation. Refer to listed references for standard methods employed in pour plate, spread plate and membrane filter procedures.[3,6] 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).

For melting bottled media: Autoclave at 121°C. for 1-3 minutes or until melted. Alternatively, a covered, boiling waterbath (100°C.) can be used. There should be enough water in the waterbath to reach the media line. A covered waterbath will help to reach and maintain the temperature. Heat in waterbath until melted. Cool media to 45-50°C. and dispense as desired.

Irradiated Media: To reduce the potential for cross-contamination, it is strongly suggested users of irradiated media use appropriate gowning and glove procedures, designated aseptic processing areas, appropriate sporicidal disinfectants, and environmental monitoring procedures to reduce the likelihood of accidental contamination of the media during use.

Membrane Filter Method:

For more detailed instructions on sampling and membrane filtration procedures, consult Standard Methods for the Examination of Water and Wastewater.[3]

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.

Pour Plate Method:

1. After autoclaving, cool media to 45-50°C. Maintain in a 45-50° waterbath until ready to pour.

2. Prepare decimal dilutions in sterile diluent to obtain 30-300 CFU per plate.

3. Place a 1ml inoculum into a sterile petri plate.

4. Aseptically pour approximately 18ml of the cooled media (45-50°C.) over the inoculum. Carefully swirl the plate to mix the inoculum evenly.

5. Allow plate(s) from step 4 to solidify.

6. 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.[3,6]

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.

The pour plate method is not highly recommended because the recovery of injured bacteria may be lessened by the heat of the media at 45°C. 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.

For Cat. no. W530, if removing the Mylar bag and storing plates long-term in a controlled environment, 2-8°C storage conditions are recommended to prevent dehydration of the medium prior to use.

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, 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	
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
Pseudomonas aeruginosa ATCC® 27853	J	1-3 days	20-25°C	Aerobic	Growth
Bacillus spizizenii ATCC® 6633	J	1-3 days	35°C	Aerobic	Growth

For Cat. no. W530: 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^{-6} following ANSI/AAMI/ISO 11137.

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

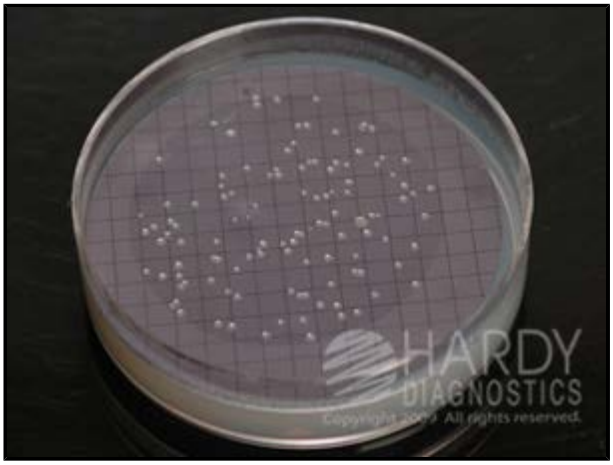
USER QUALITY CONTROL

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.

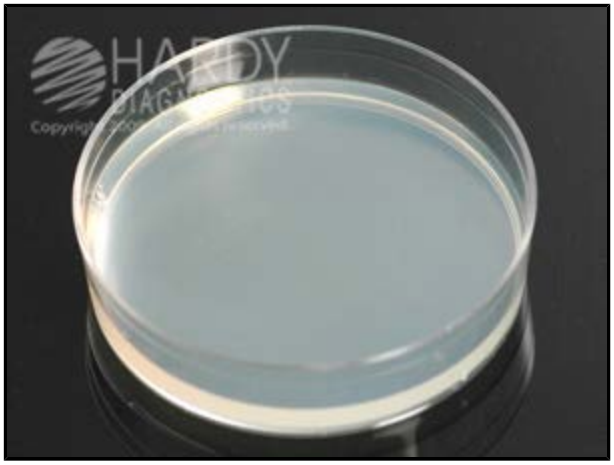
PHYSICAL APPEARANCE

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

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



Escherichia coli (ATCC® 8739) filtered through a black membrane (Cat. no. A045R047A) and growing on R2A Agar (Cat. no. G03). Incubated aerobically for 24 hours at 35°C.



Uninoculated plate of R2A Agar (Cat. no. G03).

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.

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