PRODUCT INFORMATION

Product Type: Tubes (12ml)

Cat No. TT260/BL - R-2 AGAR

Intended Use:

R2A Agar (Reasoner's 2A Agar) is a low-nutrient, solid culture medium specifically developed for the enumeration and recovery of heterotrophic bacteria, especially those present in treated potable water and other environments where bacteria may be stressed or slow-growing.

Principles and uses:

R2A Agar is a low nutrient medium, and in combination with a lower incubation temperature and longer incubation time, stimulates the growth of stressed and chlorine-tolerant bacteria. Nutritionally rich media support the growth of fast-growing bacteria, and may suppress slow growing or stressed bacteria found in treated water. When compared with Tryptone Glucose Yeast Extract Agar or Plate Count Agar (Standard Methods Agar), R2A Agar reported improved recovery of stress and chlorine-tolerant bacteria from drinking water systems. R2A Agar is recommended in standard methods for pour plate, spread plate, and membrane filter methods for heterotrophic plate counts.

Low-nutrient environment:

The limited nutrient content prevents fast-growing bacteria from outcompeting and suppressing slow-growing or stressed bacteria, enabling a more accurate recovery of total heterotrophic populations in water samples.

Enhanced bacterial recovery:

The inclusion of starch and sodium pyruvate aids in the revival and repair of injured or chlorine-tolerant bacteria, which may not grow well on richer media.

Recommended usage:

R2A Agar is cited in various international standards (e.g., US EPA, European Pharmacopoeia, APHA) for water quality assessment using techniques such as pour plate, spread plate, and membrane filtration.

Applications

Enumeration of heterotrophic bacteria in drinking water: Especially suitable for monitoring water treatment efficacy or contamination events.

Recovery of stressed or chlorine-tolerant bacteria: Important in water systems where disinfectants may have damaged microbial cells.

E**xtended/lower temperature incubation:** Designed for incubation over longer periods (typically 5–7 days) and at lower temperatures (20–28°C), which further supports the recovery of slow-growing environmental bacteria not detected on richer media.

Test Procedure

1. Prepare test dilutions for heterotrophic plate count.
2. Plate the test sample and dilutions by the spread plate, pour plate, or membrane filter method. Do not exceed 1 mL of sample or dilution per spread or pour plate. The volume of test sample to be filtered for the membrane filter technique will vary.
3. Maintain proper humidity during prolonged incubation.

Limitations of the Procedure

1. Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.
2. R2A Agar is intended for use only with treated potable water.
3. Use of the pour plate method is discouraged because recovery of stressed bacteria may be compromised by the heat shock (44-46°C) and low oxygen tension that are part of the procedure.
4. Incubation time longer than indicated above may be necessary to recover additional slow-growing bacteria.

References

1. European Pharmacopoeia 9th Edition (2017)
2. Reasoner, D. J., and E. E. Geldreich. 1979. A new medium for the enumeration and subculture of bacteria from potable water. Abstracts of the Annual Meeting of the American Society for Microbiology 79th Meeting, Paper No. N7.
3. Fiksdal, L., E. A. Vik, A. Mills, and T. Staley. 1982. Non-standard methods for enumerating bacteria in drinking water. Journal AWWA. 74:313-318.
4. Kelly, A. J., C. A. Justice, and L. A. Nagy. 1983. Predominance of chlorine tolerant bacteria in drinking water systems. Abstracts of the Annual Meeting of the American Society for Microbiology 79th Meeting, Paper No. Q122.
5. Means, E. G., L. Hanami, H. F. Ridgway, and B. H. Olson. 1981. Evaluating mediums and plating techniques for enumerating bacteria in water distribution systems. Journal AWWA. 53:585-590.
6. Greenberg, A. E., L. S. Clesceri, and A. D. Eaton (eds.). 2017. Standard methods for the examination of water and wastewater, 23rd ed. American Public Health Association, Washington, D.C.
7. VanSoestberger, A. A., and C. H. Lee. 1969. Pour plates or streak plates? Appl. Microbiol. 18:1092.
8. Klein, D. A., and S. Wu. 1974. Stress: a factor to be considered in heterotrophic microorganisms enumeration from aquatic environments. Appl. Microbiol. 27:429.

Composition:

Enzymatic Digest of Casein 0.25 g/L
Enzymatic Digest of Animal Tissue 0.25 g/L
Acid Hydrolysate of Casein 0.5 g/L
Yeast Extract 0.5 g/L
Dextrose (Glucose) 0.5 g/L
Soluble Starch 0.5 g/L
Dipotassium Phosphate 0.3 g/L
Magnesium Sulfate Heptahydrate 0.05* g/L
Sodium Pyruvate 0.3 g/L
Agar 15.0 g/L
*Equivalent to 0.024 g/L Magnesium Sulfate Anhydrous.

Storage: 15°-25°C
Package contents: 30 Tubes
Appearance: clear to slightly hazy and light Amber.
pH Range: 7.0 - 7.4
Exp. Date: Printed on label and on the item.
Required materials not supplied: Laboratory equipment as required.

Warning and Precautions:

Warning and Precautions - For professional use only. Follow good microbiological lab practices while handling specimens and culture. Do not use Tubes if they show evidence of microbial contamination, discoloration, drying, cracking, or other signs of deterioration. Avoid freezing and overheating. The Tubes may be used / inoculated up to the expiration date and incubated for the recommended incubation times. After use and prior to discarding, specimen containers and all contaminated material, including the used culture media and contaminated culture containers, must be sterilized or incinerated by validated procedures. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

Hy Laboratories Ltd.
6 Menachem Plaut St., Park Tamar, Rehovot 7670606, Israel
Tel. +972.8.9366475 **Email.** hylabs@hylabs.co.il

Implementation Date: 29/12/25
Version Number: 01

Waste Disposal

After interpretation all items should be destroyed by standard incineration methods.

Performance Testing Results:

GPT: 10-100 cfu (pour plate method*).

Test	ATCC NO	Incubation Temp. (℃)	Incubation Cond.	Reaction 1
Volume 12 ml				
Staphylococcus aureus	6538	30-35 ℃	Aerobic, 24-48 hours	Growth
Bacillus subtilis	6633	30-35 ℃	Aerobic, 24-48 hours	Growth
Bacillus cereus	14579	30-35 ℃	Aerobic, 24-48 hours	Growth
Escherichia coli	8739	30-35 ℃	Aerobic, 24-48 hours	Growth
Pseudomonas paraeruginosa	9027	30-35 ℃	Aerobic, 24-48 hours	Growth
Aspergillus brasiliensis	16404	20-25 ℃	Aerobic, 72-96 hours	Growth

***Pour plate method**

The method relies on mixing a serially diluted sample with molten agar (at 40–45°C) and pouring this mixture into a sterile Petri dish.
Each viable microorganism in the sample will grow into an individual colony after incubation.
Colonies develop both on the surface and within the agar, allowing the calculation of colony-forming units (CFU) per milliliter

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