R2A AGAR (7390)

Intended Use
R2A Agar is used for the enumeration and cultivation of bacteria from potable water.

Product Summary and Explanation
R2A Agar was developed by Reasoner and Geldreich[1] for bacterial plate counts of treated potable water. 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.[1] 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 Standard Methods Agar, R2A Agar reported improved recovery of stress and chlorine-tolerant bacteria from drinking water systems.[2,3,4] R2A Agar is recommended in standard methods for pour plate, spread plate, and membrane filter methods for heterotrophic plate counts.[5]

Principles of the Procedure
Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, and Acid Hydrolysate of Casein provide nitrogen, carbon and minerals in R2A Agar. Yeast Extract is a source of vitamins and trace elements. Dextrose serves as a carbon source in the formula. Soluble Starch aids in the recovery of injured organisms by absorbing toxic metabolic by-products. Dipotassium Phosphate is used to balance the pH, and Magnesium Sulfate Heptahydrate is a source of divalent cations and sulfate. Sodium Pyruvate increases the recovery of stressed cells. Agar is the solidifying agent.

Formula / Liter
Enzymatic Digest of Casein ..... 0.25 g
Enzymatic Digest of Animal Tissue ..... 0.25 g
Acid Hydrolysate of Casein ..... 0.5 g
Yeast Extract ..... 0.5 g
Dextrose ..... 0.5 g
Soluble Starch ..... 0.5 g
Dipotassium Phosphate ..... 0.3 g
Magnesium Sulfate Heptahydrate ..... 0.05 g
Sodium Pyruvate ..... 0.3 g
Agar ..... 15 g
Final pH: 7.2 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions
1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 18.2 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications
Dehydrated Appearance: Powder is homogeneous, free flowing and light beige.

Prepared Appearance: Prepared medium is clear to slightly hazy and light beige.

Expected Cultural Response: Cultural response in R2A Agar at 35°C after 1 - 5 days incubation.

Microorganism	Response
Enterococcus faecalis ATCC® 29212	Growth
Escherichia coli ATCC® 25922	Growth
Staphylococcus aureus ATCC® 25923	Growth

The organisms listed are the minimum that should be used for quality control testing.

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:

Incubation Temperature	Minimum Incubation Time[3]	Optimal Incubation Time[3]
35°C	72 hours	5 – 7 days
20 or 28°C	5 days	7 days

Results

Count colonies on spread or pour plates demonstrating 30 - 300 colonies per plate or 20 - 200 colonies when using the membrane filter method. Compute bacterial count per mL of sample by multiplying the average number of colonies per plate by the reciprocal of the appropriate dilution.

Report counts as colony forming units (CFU) per mL and report variables of incubation such as temperature and length of time.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if it is not free flowing, or if the medium has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

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.[6,7]
4. Incubation time longer than indicated above may be necessary to recover additional slow-growing bacteria.

Packaging

R2A Agar	**Code No.**	**7390A**	**500 g**
		7390B	**2 kg**
		7390C	**10 kg**

References

1. **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.
2. **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.
3. **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.
4. **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.
5. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
6. **VanSoestberger, A. A., and C. H. Lee.** 1969. Pour plates or streak plates? Appl. Microbiol. **18**:1092.
7. **Klein, D. A., and S. Wu.** 1974. Stress: a factor to be considered in heterotrophic microorganisms enumeration from aquatic environments. Appl. Microbiol. **27**:429.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.