R-2A Agar

Intended Use
R-2A Agar is used for enumeration of heterotrophic bacteria in treated potable water.

Summary
Reasoner and Geldreich developed R-2A medium to check the bacterial count in treated potable water. They found that plate count agar does not permit the growth of many bacteria that may be present in treated potable water supplies. Results from parallel studies with spread, membrane filter, and pour plate procedures showed that R-2A medium yielded significantly higher bacterial counts than in Plate Count Agar. Low nutritional content, longer incubation time, yielded higher counts and increased detection of heterotrophic bacteria. As a tool to monitor heterotrophic bacterial populations in water treatment processes and in treated distribution water, R-2A spread or membrane filter plates incubated at 28°C for 5 to 7 days is recommended. These conditions provide adequate time for growth of slow-growing bacteria. R-2A is useful in heterotrophic plate count analyses and for subculture of bacteria isolated from potable water samples. It is used for the recovery of stressed and chlorine-tolerant bacteria from drinking water. It is recommended by APHA for enumeration of heterotrophic bacteria in water and wastewater.

Principle
Media contains low concentration of nutrients which allows the growth of slow growing bacteria without being suppressed by fast growing bacteria. Yeast extract provides a source of trace elements and vitamins. Proteose peptone provides nitrogen, vitamins, amino acids, carbon and minerals. Dextrose serves as a carbon source. Soluble starch aids in the recovery of injured organisms by absorbing toxic metabolic by-products. Sodium pyruvate increases the recovery of stressed cells. Dipotassium phosphate is used to balance the pH and provide phosphate. Magnesium sulphate is a source of divalent cations and sulphate. Agar is the solidifying agent.

Formula*

Ingredients	**g/L**
Casein Acid Hydrolysate	0.5
Proteose Peptone	0.5
Yeast Extract	0.5
Dextrose	0.5
Soluble Starch	0.5
Sodium Pyruvate	0.3
Dipotassium Phosphate	0.3
Magnesium Sulphate	0.024
Agar	15.0
Final pH (at 25°C)	7.2 ± 0.2

*Adjusted to suit performance parameters.

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of specimen
Water and Waste Water samples.

Specimen Collection and Handling
Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 18.12 g of the powder in 1000 mL purified / distilled water.
2. Mix thoroughly.
3. Heat with frequent agitation and boil for 1 minute to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
5. Cool the medium to approximately 45°C-50°C, pour into sterile petridishes.

Quality Control

Dehydrated Appearance: Cream to light yellow, homogenous, free flowing powder.
Prepared Appearance: Off-white to light yellow coloured, clear to slightly opalescent gel, forms in petridishes.
Growth Promotion Test: Growth promotion is carried out in accordance with the harmonized method of USP/EP/JP/IP and growth is observed after an incubation at 30°C-35°C for ≤ 3 days for bacteria and at 30°C-35°C and 20-25°C for ≤ 5 days for fungi.
Growth Promoting Properties: The test results observed are within the specified temperature and shortest period of time specified in the test inoculating ≤ 100 cfu of appropriate microorganism.

Organism (ATCC)	Growth	Incubation Temperature	Incubation Period
Escherichia coli (8739)	Good	30-35°C	24 Hours
Staphylococcus aureus subsp. *aureus* (6538)	Good	30-35°C	24 Hours
Pseudomonas aeruginosa (9027)	Good	30-35°C	24 Hours
Enterococcus faecalis (29212)	Good	30-35°C	24 Hours
Bacillus spizizenii (6633)	Good	30-35°C	24 Hours
Candida albicans 3147 (10231)	Good	30-35°C	24 Hours
Candida albicans 3147 (10231)	Good	20-25°C	48 Hours
Aspergillus brasiliensis WLRI 034(120) (16404)	Good	30-35°C	48 Hours
Aspergillus brasiliensis WLRI 034(120) (16404)	Good	20-25°C	72 hours

Note: For good growth - Growth obtained on test media should not differ by a factor greater than 2 from calculated value for a standardized inoculum. In case of water samples from fields it is suggested to incubate further upto 7 days to ascertain the absence of organisms.

Interpretation of Results

Count colonies promptly on spread or pour plates showing 30-300 colonies per plate using the membrane filter method. Compute bacterial count per milliliter by the following equation:

$$\text{cfu/mL} = \frac{\text{Number of colonies per plate}}{\text{Volume of test sample, mL}}$$

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions/Limitations

1. Fast growing bacteria may produce smaller size colonies on R-2A Agar than on nutritionally rich media.
2. Pour plates do not give satisfactory results.

Warranty

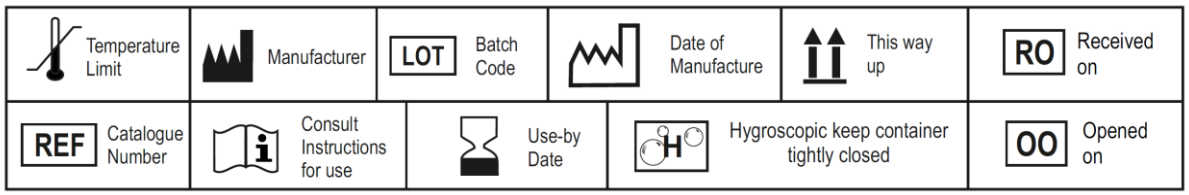
This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
2. F.P.D. Keith Ito, fourth edition. 2001. Compendium of Methods for the Microbiological Examination of Foods. Washington, D.C.: American Public Health Association.
3. Van Soestberger and Lee. 1969. Appl. Microbiol. 18:1092.
4. Klein and Wu. 1974. Appl. Microbiol. 27: 429.
5. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201180020100	Dehydrated Culture Media	100 g
201180020500	Dehydrated Culture Media	500 g
201180022500	Dehydrated Culture Media	2.5 k
203180210100	Bottle Media	100 mL
203180210250	Bottle Media	6 x 250 mL
205180260100	Ready Prepared Plate (90 mm)	100 plates



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Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.