R-2A Agar

Intended Use
R-2A Agar is recommended 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 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 and casein acid hydrolysate 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 sulfate. 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.

Directions
1. Loosen the cap.
2. Melt the medium completely in a water bath at 100°C. Do not remove the cap of the bottle while melting.
3. Cool to 45°C-50°C, mix well and pour into presterile petriplate.

Quality Control
Appearance: Light amber coloured, slightly opalescent gel.
Growth Promotion Test: Growth promotion is carried out in accordance with the harmonized method of USP/EP/JP and growth is observed after an incubation at 30°C-35°C for ≤ 3 days.
Growth Promoting Properties: The test results observed are within the specified temperature and shortest period of time, inoculating ≤ 100 cfu of the appropriate organism (at 30°C-35°C for ≤ 3 days).

Organisms (ATCC)	Growth
Escherichia coli (8739)	Good
Enterococcus faecalis (29212)	Good
Pseudomonas aeruginosa (9027)	Good
Staphylococcus aureus subsp. *aureus* (6538)	Good
Bacillus spizizenii (6633)	Good

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.
Inoculum cfu for good growth is 10-100.

Remarks

1. Do not use media bottles that exhibit any damage, cracks, microbial contamination, discoloration, drying or other sign of deterioration.
2. Ensure that the temperature of water bath is at 100°C so that the medium melts completely. Cooler water baths give rise to lumpy, uneven medium.
3. Before pouring into sterile petriplates, gently swirl the bottle to check whether the entire contents are properly mixed and melted.
4. Good laboratory practices and hazard precautions must be observed at all times.
5. After use media containers, prepared plates, sample, sample containers and other contaminated materials must be sterilized or incinerated before discarding.

Storage and Stability

1. Store the ready to use R-2A Agar at 15°C-25°C in a cool, dry place away from light.
2. Stability of the kit is as per expiry date mentioned on the label.

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.

References

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
203180210100	Bottle Media	100 mL
203180210250	Bottle Media	6 x 250 mL