R-2A Agar Plate (Triple Layer Pack, Gamma-Irradiated)

Intended Use
R-2A Agar Plate is used for heterotrophic plate court of treated potable water.

Summary
Reasoner and Geldreich developed R2A 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 R2A medium yielded significantly higher bacterial counts than did 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, R2A spread, or membrane filter plates incubated at 20°C - 28°C for 5 to 7 days is recommended. These conditions provide adequate time for growth of slow-growing bacteria. R2A 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 2-3 bacteria in water and wastewater.

Principle
Gamma Irradiated R-2A Agar Plates are triple - layer packed in stacks of five plates. The presence of an irradiation indicator enables the rapid and easy visual confirmation by the cleanroom operator that the medium is irradiated. Each pack (media and their wrappings) receives an irradiation dose between 23 and 32 kGy to guarantee that no viable contaminants are present.
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. Glucose 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 sulfate is a source of divalent cations and sulfate. Agar is a solidifying agent.

Formula*

Ingredients	**g/L**
Yeast Extract	0.5
Proteose Peptone	0.5
Casein Hydrolysate	0.5
Glucose	0.5
Soluble Starch	0.5
Sodium Pyruvate	0.3
Dipotassium Phosphate	0.3
Magnesium Sulfate Anhydrous	0.024
Bacteriological Agar	15.0

*Adjusted to suit performance parameters.

Additional Material Required
Bacteriology Incubator.

Instructions for use
1. Open the sterile pack and remove R2A Agar Plate aseptically.
2. Inoculate/streak the plate and Incubate in inverted position as per standard procedure.

Reading and interpretation
1. After incubation, observe the microbial growth and count the colonies.
2. Interpretation is assured by user.
3. User is responsible to define the action limits as per standard guidelines and alert limits on the basis of trend analysis & other relevant data.

Quality Control

Appearance: Gel with smooth and even surface without any cracks, bubbles and drying or shrinking of media.
Colour of Medium: Light yellow coloured, slightly opalescent gel in petriplates.
Quantity of Medium: 29 ± 2 g in 90 mm petriplate.
pH at 25°C±2°C: 7.2 ± 0.2
Gamma Irradiation: The above said product was Gamma Irradiated between 23KGy - 32KGy.
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 for bacteria and at 30°C-35°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.

Cultural Response:

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

Storage and Shelf Life

1. Store between 15°C-25°C to avoid water condensation. Condensation can be prevented by avoiding quick temperature shifts and mechanical stress.
2. Under optimal conditions, the medium has a shelf life of 6 months. Use before expiry mentioned on the label.

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. Environment Agency- The Microbiology of Drinking Water 2002
3. European Pharmacopoeia 2002, supplement 4.6.
4. F.P.D. Keith Ito, fourth edition. 2001. Compendium of Methods for the Microbiological Examination of Foods. Washington, D.C.: American Public Health Association.
5. Greenbreg, Trussell and Clesceri (ed) (1998) *Standard Methods for the Examination of Drinking Water and Waste*
   Water. 20th Ed. APHA, Washington DC.
6. Klein and Wu. 1974. Appl. Microbiol. 27: 429.
7. Reasoner and Geldreich (1985). *Appl. Environ. Microbiol*. 49, 1.
8. Van Soestberger and Lee. 1969. Appl. Microbiol. 18:1092.
9. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product	Pack Size
205180260100	R-2A Agar Plate	100 Plates