R-2A Agar (Agar Medium S) EP

Intended Use
R-2A Agar (Agar Medium S) is used for enumeration of heterotrophic bacteria in treated potable water in compliance with EP.

Summary
R2A Agar is a medium with a low nutrient content, which, in combination with a low incubation temperature and an extended incubation time, is especially suitable for the recovery of stressed and chlorine-tolerant bacteria from drinking water. The nutrient medium conforms with recommendations of the standard methods (US-EPA), the European Pharmacopeia (2014) and the British Pharmacopeia for the examination of water.

Principle
The low concentration of yeast extract, casein hydrolysate, proteose peptone and glucose allows a wide spectrum of bacteria to grow without the fast-growing bacteria suppressing the slow-growing species, such as would be the case on richly nutritious media like e.g. Plate Count Agar. The content of starch and pyruvate allows particularly the injured bacteria to grow again more quickly.

Formula*

Ingredients	g/L
Yeast Extract	0.5
Proteose Peptone	0.5
Casein Hydrolysate	0.5
Glucose	0.5
Starch	0.5
Dipotassium Hydrogen Phosphate	0.3
Magnesium Sulfate Anhydrous	0.024
Sodium Pyruvate	0.3
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 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 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 petriplates.

Quality Control
Dehydrated Appearance: Cream to light yellow coloured, homogenous, free flowing powder.
Prepared Appearance: Off white to light yellow coloured, slightly opalescent gel forms in petriplates.
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.

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
Escherichia coli (8739)	Good
Pseudomonas aeruginosa (9027)	Good
Bacillus subtilis (6653)	Good
Enterococcus faecalis (29212)	Good
Staphylococcus aureus (6538)	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. In case of water samples from fields it is suggested to incubate further upto 7 days to ascertain the absence of organisms.

Performance and Evaluation

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

1. Directions
2. Storage
3. Expiry

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. Eaton, A. D., Clesceri L.S. and Greenberg A.E. (1995). Standard methods for the examination of water and wastewater, 19th. Ed. APHA, Washington D.C.
2. European Pharmacopoeia 8.0 (2014) Monographs: Water for injections; highly purified; Water purified.
3. Fiksdal, L., Vik E.A., Mills A. and Staley T. (1982). Non-standard methods for enumerating bacteria in drinking water. Journal AWWA. 74: 313-318.
4. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201180030500	Dehydrated Culture Media	500 g