R-2A Broth

Intended Use
R-2A Broth is used for cultivation and maintenance of heterotrophic bacteria from potable waters.

Summary
The total bacterial count of drinking water is determined by plate count on a nutritionally rich medium. However, all organisms present are not able to grow on them, either because they are slow growers or because they can't grow on that media. For this reason, a nutritionally reduced medium was described. R-2A Agar is a modification of this medium. R-2A Broth enables better recovery of these bacteria from treated waters under different incubation conditions. Many bacteria from natural waters, which contain limited nutrients at ambient temperature, grow best on the media with less nutrient levels. They grow better at the temperatures below the routine laboratory incubation temperatures of 35°C to 37°C.

Principle
This medium contains casein acid hydrolysate, yeast extract, proteose peptone as source of essential growth factors required for metabolism of the bacteria. Dextrose is the energy source. Starch acts as a neutralizer that neutralizes any toxic metabolites, if present. Phosphate buffers the medium while sodium pyruvate supplies additional nutrition. Magnesium sulphate serves as a source of ions. Due to the presence of the above-mentioned ingredients these media allow the growth of stressed and chlorine tolerant bacteria present in treated waters.

Formula*

Ingredients	**g/L**
Casein Acid Hydrolysate	0.5
Yeast Extract	0.5
Proteose peptone	0.5
Dextrose	0.5
Starch, Soluble	0.5
Dipotassium Phosphate	0.3
Magnesium Sulphate	0.024
Sodium Pyruvate	0.3
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 3.12 g of the powder in 1000 mL purified / distilled water.
2. Heat if necessary, to dissolve the powder completely.
3. Dispense into tubes. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle. DO NOT OVERHEAT.

Quality Control

Dehydrated Appearance: Cream to light yellow, homogenous, free flowing powder.
Prepared Appearance: Yellow to light yellow coloured, clear to slightly opalescent solution forms in tubes.
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 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**
Escherichia coli (25922)	Good
Enterococcus faecalis (29212)	Good
Salmonella enterica subsp. *enterica* serovar *Enteritidis* (13076)	Good
Candida albicans 3147 (10231)	Good

Note: Inoculum cfu for good growth is 10-100. 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. Reasoner and Geldreich, 1985, Appl. Environ. Microbiol., 49:1.
2. Stark and McCoy. 1938. Zentralbl. Bacteriol. Parasitenkd. Infectionskr. Hyg. Abt.2 98 : 201
3. Collins and Willoughby, 1962, Arch. Microbiol., 43:294.
4. Greenberg A. E., Trussell R. R. and Clesceri L. S. (Eds.), 1985, Standard Methods for the Examination of Water and Wastewater, 16th ed., APHA, Washington, DC.
5. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	**Product description**	**Pack Size**
201180010500	Dehydrated Culture Media	500 g
201180012500	Dehydrated Culture Media	2.5 k
203180220010	Ready Prepared Tube	25 x 10 mL
203180220100	Bottle Media	100 mL