Instructions For Use
ENGLISH

R2A Agar

Medium for enumerating heterotrophic organisms
in water, according to the EP and APHA.

DESCRIPTION

R2A Agar is a low nutrient medium used for microbial monitoring of treated potable water.

This medium is recommended by the European Pharmacopoeia (EP) and the American Public Health Association (APHA) for the examination of water.

TYPICAL FORMULA	(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 Sulphate, Anhydrous	0.024
Sodium Pyruvate	0.3
Agar	15.0
Final pH 7.2 ± 0.2 at 25°C	

METHOD PRINCIPLE

Yeast extract is a source of vitamins, particularly of B-group. Proteose peptone and casein hydrolysate provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Glucose is the fermentable carbohydrate. Starch aids in the recovery of injured organisms by absorbing toxic metabolic by-products. Dipotassium phosphate maintains the osmotic balance the medium. Magnesium sulphate is a source of divalent cations and sulfate. Sodium pyruvate increases the recovery of stressed cells. Agar is the solidifying agent.

PREPARATION

Dehydrated medium	Suspend 18.1 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Sterilize in autoclave at 121°C for 15 minutes.
Medium in bottles	Melt the content of the bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into Petri dishes.

TEST PROCEDURE

1. Perform serial dilutions of the water sample in order to achieve a suitable colony count and prepare two sets of plates for each dilution.
2. Inoculate the medium by pour plating, spread plating or membrane filtration method. RODAC plates can also be used directly on surfaces to asses the efficacy of cleaning and sanitization procedures as part of the overall microbiological monitoring program.
3. Incubate aerobically one set of plates at 30-35°C for 3-5 days and the other set at 20-25°C for 5-7 days.

NB. Incubation conditions may vary depending on the organisms under study. Generally, a lower incubation temperature and longer incubation period, stimulates the growth of stressed and chlorine-tolerant bacteria.

INTERPRETING RESULTS

Report the count as CFU/ml of sample allowing for dilution factors and noting incubation time and temperature.

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, light beige.
Prepared medium: slightly opalescent with a slight precipitate, light amber.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles and prepared plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in bottles: 2 years.
Ready-to-use plates: 6 months.

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QUALITY CONTROL

Plates are inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: ≤ 100 CFU
Incubation conditions: aerobic, 32.5 ± 2.5°C for up to 3 days (bacteria) and at 22.5 ± 2.5°C for 5-7 days (yeasts and moulds).

QC Table.

Microorganism		Growth
*Pseudomonas aeruginosa**	ATCC® 9027	Good
*Bacillus subtilis**	ATCC® 6633	Good
Enterococcus faecalis	ATCC® 19433	Good
Escherichia coli	ATCC® 8739	Good
Staphylococcus aureus	ATCC® 6538	Good
Candida albicans	ATCC® 10231	Good
Aspergillus brasiliensis	ATCC® 16404	Good

(*) Pharmacopoeia growth promotion.

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use.
The product is intended for professional use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. European Pharmacopoeia 7.0 (2009) Water For Injections / Water, Highly Purified.
2. Clesceri, L.S., A.E. Greenberg, and A.D. Eaton (1998) Standard Methods for the Examination of Water and Wastewater. 20th ed. American Public Health Association, Washington, D.C.
3. Reasoner, D.J. and E.E. Geldreich (1985) Appl. Environ. Microbiol. 49:1-7.

PRESENTATION		Contents	Ref.
R2A Agar	90 mm ready-to-use plates	20 plates	10019
R2A Agar	60 mm ready-to-use plates	20 plates	163672
R2A Agar	55 mm ready-to-use RODAC plates	20 plates	15354
R2A Agar	Bottles (screw cap)	6 x 500 ml bottles	463110
R2A Agar	Bottles (wide neck)	6 x 500 ml bottles	472580
R2A Agar	Bottles (screw cap)	6 x 100 ml bottles	402580
R2A Agar	Dehydrated medium	500 g of powder	610129
R2A Agar	Dehydrated medium	100 g of powder	620129

TABLE OF SYMBOLS

LOT Batch code	Keep away from sunlight	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse

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