

Technical Data Sheet

R2A Agar

Ordering number: 1.00416.0500

R2A Agar is a nutrient medium for the determination of the heterotrophic total bacterial count in drinking water.

R2A Agar is a medium with a low nutrient content, which, in combination with a low incubation temperature and an extended incubation time, is specially 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) and the European Pharmacopeia (2014) for the examination of water.

Mode of Action

The low concentration of yeast extract, casein hydrolysate, 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.

Typical Composition

Yeast Extract	0.5 g/l
Proteose Peptone	0.5 g/l
Casein Hydrolysate	0.5 g/l
Glucose	0.5 g/l
Soluble Starch	0.5 g/l
Sodium Pyruvate	0.3 g/l
K ₂ HPO ₄	0.3 g/l
MgSO ₄ , anhydrous	0.024 g/l
Agar-Agar	15 g/l

Preparation

Suspend 18.2 g in 1 l demineralized water and heat in a boiling water bath or flowing steam until the medium has completely dissolved. Autoclave for 15 min at 121 °C. Cool to 45–50 °C and pour into sterile Petridishes.

The appearance of the medium is clear to slightly turbid and yellowish.

The pH value at 25 °C is in the range of 7.0-7.4.

Experimental Procedure and Evaluation

The R2A agar plates are designed for the determination of the total microbial count in water for injections in bulk, highly purified water and purified water in bulk.

For the total viable aerobic count 200 ml water (for water for injection in bulk and for highly purified water) are filtered through a membrane ($\leq 0.45 \mu\text{m}$). The filter is subsequently incubated on R2A agar. The medium is incubated under aerobic conditions for 5 days at 30-35 °C.

If an incubation time of more than 3 days is used, the plates should be protected from dehydration.

Incubation temperature	Minimum incubation time	Maximum incubation time
35 °C	3 days	5-7 days
20 or 28 °C	5 days	7 days

According to the EP water for injections in bulk and highly purified water must contain less than 10 colony forming units (CFUs) per 100 ml, purified water in bulk must contain less than 100 CFUs per ml.

Finally the number of CFU per plate is examined.

Grown colonies are recommended to be identified.

Storage

The product can be used for sampling until the expiry date if stored upright, protected from light and properly sealed at +15 °C to +25 °C.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed.

The prepared plates can be stored for 4 weeks under correct storage conditions (+2 to +8 °C, protected from light and dehydration).

Disposal

Please mind the respective regulations for the disposal of used culture medium (e.g. autoclave for 20 min at 121 °C, disinfect, incinerate etc.).

Quality Control

Control Strains	ATCC #	Inoculum CFU	Incubation	Expected Results
<i>Escherichia coli</i>	8739	10-100	24 h at 35 °C	Recovery rate $\geq$ 70 %
			72 h at 20-25 °C	
<i>Pseudomonas aeruginosa</i>	9027	10 - 100	24 h at 35 °C	Recovery rate $\geq$ 70 %
			72 h at 20-25 °C	
<i>Pseudomonas aeruginosa</i>	27853	10 - 100	24 h at 35 °C	Recovery rate $\geq$ 70 %
			72 h at 20-25 °C	
<i>Staphylococcus aureus</i>	6538	10-100	24 h at 35 °C	Recovery rate $\geq$ 70 %
			72 h at 20-25 °C	
<i>Bacillus subtilis</i>	6633	10-100	24 h at 35 °C	Recovery rate $\geq$ 70 %
			72 h at 20-25 °C	
<i>Pseudomonas fluorescens</i>	17386	10-100	72 h at 20-25 °C	Recovery rate $\geq$ 50 %
<i>Methylobacterium extorquens</i>	15911 (NBRC #)	10-100	72 h at 20-25 °C	Recovery rate $\geq$ 50 %

Please refer to the actual batch related Certificate of Analysis.

Literature

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.

European Pharmacopoeia 8.0 (2014) Monographs: Water for injections; Water, highly purified; Water purified.

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.

Japanese Pharmacopoeia 16th edition (2011), Section G8 4.4.2

Means, E.G., Hanami L., Ridgway H.F. and Olson B.H. (1981). Evaluating mediums and plating techniques for enumerating bacteria in water distributing systems. Journal AWWA. **53**: 585-590.

Reasoner, D.J. and Geldreich E.E. (1979). A new medium for the enumeration and subculture of bacteria from potable water. Abstracts of the Annual Meeting of the American Society for Microbiology 79th Meeting, Paper No. N7.

Ordering Information

Product	Cat. No.	Pack size
R2A Agar	1.00416.0500	500 g

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