# 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 hydrolisate, 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 |
| $K_2HPO_4$ | 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 (≤ 0.45 µm). 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 |
|---|---|---|---|---|
| *Escherichia coli* | 8739 | 10-100 | 24 h at 35 °C<br>72 h at 20-25 °C | Recovery rate ≥ 70 % |
| *Pseudomonas aeruginosa* | 9027 | 10 - 100 | 24 h at 35 °C<br>72 h at 20-25 °C | Recovery rate ≥ 70 % |
| *Pseudomonas aeruginosa* | 27853 | 10 - 100 | 24 h at 35 °C<br>72 h at 20-25 °C | Recovery rate ≥ 70 % |
| *Staphylococcus aureus* | 6538 | 10-100 | 24 h at 35 °C<br>72 h at 20-25 °C | Recovery rate ≥ 70 % |
| *Bacillus subtilis* | 6633 | 10-100 | 24 h at 35 °C<br>72 h at 20-25 °C | Recovery rate ≥ 70 % |
| *Pseudomonas fluorescens* | 17386 | 10-100 | 72 h at 20-25 °C | Recovery rate ≥ 50 % |
| *Methylobacterium extorquens* | 15911 (NBRC #) | 10-100 | 72 h at 20-25 °C | Recovery rate ≥ 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.

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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.

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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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