

## Technical Data Sheet

# R2A Agar chromogenic - LI

Ordering number: 1.46049.0020

R2A Agar chromogenic - LI in 90 mm settle plates is designed for the determination of the total microbial count in water for injections in bulk, highly purified water and purified water in bulk.

Ten settle plates each with a diameter of 90 mm are single-bagged in transparent, hydrogen peroxide impermeable sleeves (non-irradiated). The sleeves consist of polypropylene with a barrier of PE-EVOH-PE.

The formulation of the basic medium (R2A Agar) is prepared according to the recommendations of the current European Pharmacopoeia (EP, Water for injections) and supplemented with a chromogenic agent.

### Mode of Action

R2A Agar (Reasoner's 2A agar) is especially designed to promote the growth of aerobic, heterotrophic microorganisms which are adapted to low nutrient environments such as water. The low amount of nutrients in the agar allows the growth of aerobic, heterotrophic microorganisms which are adapted to a low nutrient content.

The chromogenic agent will cause a red color of the colonies of gram-negative and gram-positive bacteria as well as a number of yeasts. Thereby the evaluation of the plates is facilitated. Molds are not affected by the chromogenic agent.

### 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   |
| Starch                          | 0.5 g/l   |
| K <sub>2</sub> HPO <sub>4</sub> | 0.3 g/l   |
| MgSO <sub>4</sub>               | 0.024 g/l |
| Sodium Pyruvate                 | 0.3 g/l   |
| Agar                            | 15 g/l    |
| Chromogenic Supplement          |           |

The appearance of the medium is clear and whitish. The pH value is in the range of 7.0-7.4. The medium can be adjusted and/or supplemented according to the performance criteria required.

## Application and Interpretation

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.

The recovery of a number of tested microorganisms has not been lowered by the chromogenic agent. Recoveries of different test strains on the classic R2A Agar - LI (article number 146075) and R2A Agar chromogenic - LI (article number 146049) are shown in the following table:

### Recoveries of different test strains on classic R2A Agar and R2A Agar chromogenic

| Test strain                    | ATCC # | Recovery (%)<br>R2A Agar - LI | Recovery (%)<br>R2A Agar chromogenic - LI |
|--------------------------------|--------|-------------------------------|---|
| <i>Acinetobacter lwoffii</i>   | 15309  | 61                            | 71  |
| <i>Acinetobacter baumannii</i> | 19606  | 103                           | 114                                       |
| <i>Aeromonas hydrophila</i>    | 7966   | 111                           | 97  |
| <i>Aeromonas caviae</i>        | 15467  | 90                            | 99  |
| <i>Bacillus subtilis</i>       | 6633   | 107                           | 124                                       |
| <i>Burkholderia cepacia</i>    | 25416  | 116                           | 78  |
| <i>Escherichia coli</i>        | 8739   | 107                           | 92  |
| <i>Pseudomonas aeruginosa</i>  | 27853  | 83                            | 78  |
| <i>Pseudomonas aeruginosa</i>  | 9027   | 110                           | 84  |
| <i>Ralstonia pickettii</i>     | 27511  | 115                           | 120                                       |
| <i>Staphylococcus aureus</i>   | 6538   | 111                           | 102                                       |

Each plate is provided with a label including a data matrix code for paperless plate identification. The code consists of a two-dimensional 20-digit serial number, which harbors the following information:

digits 1-3: here code 776 (corresponds to article 146049); digits 4-9: lot number; digits 10-14: batch specific individual number; digits 15-20: expiration date (YY/MM/DD).

Please check each agar plate before using it on sterility and pay attention to aseptic handling in order to avoid false positive results.

For the total viable aerobic count 200 ml water (for water for injection in bulk and for highly purified water) is 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.

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 colony forming units per ml.

Finally the number of CFU per plate is examined.

For the ongoing identification of isolated microorganisms R3A-Agar (article number 146074) or Plate-Count-Agar (article number 146269) can be used for subcultivation.



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## Storage and Shelf Life

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.

Condensation can be prevented by avoiding quick temperature shifts and mechanical stress.

The testing procedures as described on the CoA can be started up to the expiry date printed on the label.

## 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 Result  |
|-------------------------------|--------|--------------|---------------------|--|
| <i>Escherichia coli</i>       | 8739   | 10-100       | 44-48 h at 30-35 °C | 70-200 %; good growth; reddish, medium-sized colonies          |
| <i>Pseudomonas aeruginosa</i> | 9027   | 10-100       | 44-48 h at 30-35 °C | 70-200 %; good growth; reddish, small colonies                 |
| <i>Bacillus subtilis</i>      | 6633   | 10-100       | 44-48 h at 30-35 °C | 70-200 %; good growth; pink-reddish, large, flat, dry colonies |
| <i>Burkholderia cepacia</i>   | 25416  | 10-100       | 44-48 h at 30-35 °C | 70-200 %; good growth; reddish, small colonies                 |
| <i>Ralstonia pickettii</i>    | 27511  | 10-100       | 44-48 h at 30-35 °C | 70-200 %; good growth; reddish, small colonies                 |
| <i>Candida albicans</i>       | 10231  | 50-200       | 70-74 h at 30-35 °C | 70-200 %; good growth; reddish, small, dry, arched colonies    |

Please refer to the actual batch related Certificate of Analysis.

## Literature

Greenberg, A.E., Clesceri L.S. and Eaton, A.D. (1992): Standard methods for the examination of water and wastewater, 18<sup>th</sup> ed. American Public Health Association, Washington, D.C.

Hedderich, H. and Haberer, K. (2009): A chromogenic R2A-Agar for the facilitated detection of heterotrophic bacteria in water samples. Posterpräsentation zur 61. Jahrestagung der Deutschen Gesellschaft für Hygiene und Mikrobiologie.

Reasoner, D.J. and Geldreich, E.E. (1985): A new medium for the enumeration and subculture of bacteria from potable water. Appl. Environ. Microbiol. 49: 1-7.

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



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## Ordering Information

| Product  | Cat. No.     | Pack size |
|--|--------------|-----------|
| R2A Agar chromogenic - LI  | 1.46049.0020 | 20 pcs    |
| R2A Agar Culture Media   | 1.00416.0500 | 500g      |
| R2A Agar - LI  | 1.46075.0020 | 20 pcs    |
| R2A Agar - LI  | 1.46075.0120 | 120 pcs   |
| R3A Agar - LI  | 1.46074.0020 | 20 pcs    |
| Plate Count Agar   | 1.46269.0020 | 20 pcs    |
| Plate Count Agar   | 1.46269.0100 | 100 pcs   |
| GranuCult™ Plate Count Agar acc. ISO 4833, ISO 17410 and FDA-BAM | 1.05463.0500 | 500 g     |

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