

## Technical Data Sheet

### HEIMPLATE™ Chromogenic R2A

Ordering number: 1.46049.0020

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.

#### General

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 (g/l)

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.

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## 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 HEIMPLATE™R2A and HEIMPLATE™Chromogenic R2A are shown in the following table:

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

Test strain	ATCC #	Recovery (%) HEIMPLATE™ R2A	Recovery (%) HEIMPLATE™ Chromogenic R2A
<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 picketii</i>	27511	115	120
<i>Staphylococcus aureus</i>	6538	111	102

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 or Plate- Count-Agar can be used for subcultivation.

## Storage and Shelf Life

The product can be used for sampling until the expiry date if stored upright, protected from light

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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, 18th 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
HEIMPLATE™ Chromogenic R2A	1.46049.0020	20 pcs
HEIMPLATE™ R2A	1.46075.0020	20 pcs
HEIMPLATE™ R2A	1.46075.0120	120 pcs
HEIMPLATE™ R3A	1.46074.0020	20 pcs
GranuCult™ Plate Count Agar acc. ISO 4833, ISO 17410 and FDA-BAM	1.05463.0500	500 g

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