TECHNICAL DATA SHEET

R2A AGAR

ENUMERATION OF MICROORGANISMS IN WATER

1 INTENDED USE

The R2A (Reasoner's 2A) agar is a medium destined for the enumeration of total viable aerobic microorganisms in treated water such as purified water, highly purified water and water for injectable preparation.
The R2A medium is in accordance with European Pharmacopoeia (former medium S).

2 PRINCIPLES

Present in small quantity, the nutritive elements permit the development of stressed microorganisms.

The combination of mineral salts maintains the osmotic balance.

The presence of sodium pyruvate activates the bacterial metabolism.

3 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media :

- Yeast extract ..... 0,5 g
- Proteose peptone ..... 0,5 g
- Acid hydrolysate of casein ..... 0,5 g
- Glucose ..... 0,5 g
- Starch ..... 0,5 g
- Dipotassium phosphate ..... 0,3 g
- Magnesium sulfate, anhydrous ..... 0,024 g
- Sodium pyruvate ..... 0,3 g
- Bacteriological agar ..... 15,0 g

pH of the ready-to-use media at 25 °C : 7,2 ± 0,2.

4 PREPARATION

- Dissolve 18,1 g of dehydrated media (BK179) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense in tubes or vials.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool and maintain the media in a molten state at 44-47 °C.
- Pour into sterile Petri plates (55 mm diameter).
- Let solidify on a cold, flat surface.

✓ **Reconstitution :**
18,1 g/L

✓ **Stérilisation :**
15 min à 121 °C

Use of ready-to-melt media

Melt the media (if it was prepared in advance) or with the ready-to-melt media (BM183) melt for the minimum amount of time necessary to achieve total liquefaction.

5 INSTRUCTIONS FOR USE

- Aseptically filter through a membrane a known volume of the sample to test.
- Deposit the membrane on the surface of the agar, filtered side up and making sure that the membrane and agar are in close contact. The plates should be brought to room temperature before use.
- Incubate at 30-35 °C for at least 5 days.

✓ **Inoculation :**
Membrane filtration

✓ **Incubation :**
At least 5 days at 30-35 °C

6 RESULTS

Enumerate the plates containing less than 150 cfu per membrane filter.

7 QUALITY CONTROL

Dehydrated media : white-cream powder, free-flowing and homogeneous.
Prepared media : amber agar.

Typical culture response after 48 hours of incubation at 30-35 °C, inoculum ≤ 100 microorganisms

Microorganisms		Growth (Productivity Ratio : P_R)
Bacillus subtilis	WDCM 00003	$P_R \geq 50$ %
Pseudomonas aeruginosa	WDCM 00026	$P_R \geq 50$ %

8 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.
Ready-to-melt media in vials : 2-25 °C.
The expiration dates are indicated on the labels.

Prepared media in tubes or vials (*) : 180 days at 2-25 °C.
Prepared media in plates (*) : 30 days at 2-8 °C.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

9 PACKAGING

Dehydrated media
500 g bottle BK179HA

Ready-to-melt media
10 x 100 mL vials BM18308

10 BIBLIOGRAPHY

D. J. Reasoner and E. E.,Geldreich. A New Medium for the Enumeration and Subculture of Bacteria from Potable Water. Appl Environ Microbiol. Jan 1985; 49(1): 1–7.

Pharmacopée européenne. Eau purifiée.

Pharmacopée européenne. Eau hautement purifiée.

Pharmacopée européenne. Eau pour préparation injectables.

11 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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