RAPID'*P. aeruginosa* Agar for water testing

356-4900
356-3984

SCOPE

RAPID'*P.aeruginosa* is a selective chromogenic culture medium for the direct enumeration (without confirmation) of *Pseudomonas aeruginosa* using the membrane filtration method.
It is particularly suitable for the testing of water for human consumption, in particular bottled water and untreated water such as water from wells, lakes and streams wherein the commensal water flora is abundant and for which the standardised medium is non-specific for *P. aeruginosa.*

AFNOR CERTIFICATION according to protocol AFNOR Rev 1

The *RAPID'P.aeruginosa* method for water testing is NF Validation certified as an alternative method to the ISO 16266 standard for the counting of Pseudomonas aeruginosa at 36°C **in water with a low MES content, including bottled water**, according to the Validation Reference frame "Protocol for Validation of an alternative commercial method with respect to a reference method (revision 1)" (adopted by AFNOR Certification on 10/05/2010), under the certificate no.:
BRD 07/21 – 04/12.
This certificate is available from Bio-Rad representative or AFNOR Certification.

BRD 07/21 – 04/12
Water analysis methods
Certified analytical performance characteristics
www.afnor-validation.org

NORMATIVE REFERENCE(S)

WATER

• **EN ISO 16266 (08/2008)**: Water quality – Detection and enumeration of *Pseudomonas aeruginosa* - Membrane filtration method.

• **NF T90-461/A2 (May 2007):** Water quality – Microbiology – Quality control of culture media.

PRINCIPLE

The principle of RAPID'*P.aeruginosa* medium is based on the detection of an enzymatic activity typical of *P.aeruginosa*. Under its action, a specific chromogenic substrate is cleaved leading to the formation of a blue to blue-green precipitate on the *Pseudomonas aeruginosa* colonies.

The selective mixture makes it possible to inhibit the majority of interfering flora, very important in water, in particular *Pseudomonas* non *aeruginosa*.
Other microorganisms may however show growth. Their colonies appear transparent or pigmented yellow-green and are easily distinguishable from those of *Pseudomonas aeruginosa*.

PRESENTATION

- **RAPID'*P.aeruginosa* Agar_prepoured**
  55 mm x 20 dishes **code 356-3984**

- **RAPID'*P.aeruginosa* Agar_dehydrated**
  500 g **code 356-4900**

STORAGE/VALIDITY/BATCH

- Ready to use: +2-8°C protected from light.
- Dehydrated: +2-8°C, bottle carefully closed in a cool, dry place.
- Petri dishes prepared by the user: 15 days at +2-8°C, protected from light
- The expiry date and the batch number are stated on the packaging.

THEORETICAL FORMULA

RAPID'*P.aeruginosa* medium

Nutritive mixture	14 g
Buffer system	2.15 g
Chromogenic substrate	0.13 g
Selective mixture	0.11g
Agar	10 g
Distilled water	1000 ml

Final pH (25°C) = 7.1 ± 0.2

OTHER REQUIRED PRODUCTS (NOT SUPPLIED)

Sterile distilled water

Bio-Rad, 3 boulevard Raymond Poincaré • 92430 Marnes-la-Coquette
Tel. : +33 (0)1 47 95 60 00 • Fax : +33 (0)1 47 41 91 33
www.foodscience.bio-rad.fr

RAPID' *P. aeruginosa* Agar
for water testing

REQUIRED EQUIPMENT (NOT SUPPLIED)
(non-exhaustive list)

- Agitator-homogeniser
- Filtration device
- Forceps for the handling of membranes
- Sterile pipettes (1 ml, 5 ml….)
- Sterile Petri dishes (Ø = 55 mm)
- Sterile filtration membranes (Ø = 47 mm, 0.45 µm Millipore HAWG 047 Type HA)
- Water bath with a precision of ±1°C
- Ovens or thermostated enclosures with a precision of ± 1°C
- All standard laboratory equipment

PREPARATION OF THE MEDIUM

Always stir before each use.

- Take up 26.4 grams of powder in a litre of distilled water. Mix until a homogeneous suspension is obtained. While stirring, heat slowly and bring to boiling until complete dissolution.
- Sterilise in the autoclave at 121 ± 3°C for 15 minutes.
- Cool to approximately 44-47°C.
- Distribute in sterile Petri dishes (Ø 55 mm, 3 mm to 5 mm thickness), 8 to 10 ml per dish.
- Allow to solidify on a cool horizontal surface.

Reconstitution level: 26.4 g/l
500 grams of powder make possible the concoction of 18.9 litres of medium.

PROTOCOL

• Sample preparation

To be carried out in accordance with the standard of the product concerned.

• Seeding and incubation

- Filter under sterile conditions through a membrane the volume of water to be analysed according to the origin of the sample (e.g., 250 ml for a sample of bottled water)
- Place the membrane, cross-hatched surface up, on the surface of the medium previously brought to room temperature, taking care that the membrane-agar contact is complete.
- Turn the dishes over and incubate them at 36 ± 2°C for **22-30 hours.**

READING AND INTERPRETATION

• Counting of colonies (CFU)

After incubation, carry out the reading on the top of the membrane. Count as *Pseudomonas aeruginosa* all colonies blue to blue green, regardless of their shape.

Note: Retain only the dishes containing less than 50 characteristic colonies and less than 100 colonies in total.

• Expression of results/Calculations

Put down the number of total *Pseudomonas aeruginosa* per unit volume of filtered sample.

PRECAUTIONS FOR USE

- The dehydrated format contains Triclosan at 0.028%, which is classified as a product dangerous to the environment:

N – Dangerous to the environment

- **R51/53**: Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
- **S57**: Use appropriate containment to avoid environmental contamination.
- **S60**: This material and its container must be disposed of as hazardous waste.
  The safety data sheet is available on request.
- Avoid trapping air bubbles underneath the membrane during its placement on the agar. Poor membrane-agar contact may lead to an erroneous result. If necessary, gently and carefully flatten the membrane with the forceps.
- Comply with Good Laboratory Practice.

PERFORMANCE CHARACTERISTICS/QUALITY CONTROL OF THE TEST

The performance characteristics of the culturing are tested by means of the following strains:

STRAINS	Results after 22-30 h of incubation at 36°C	
	GROWTH	COLOUR
P. aeruginosa ATCC 9027	+	blue
E. faecalis CCM 2541	Inhibition	NA

*Fertility yield: R = [66% - 150%]
NA: Not Applicable

Bio-Rad, 3 boulevard Raymond Poincaré • 92430 Marnes-la-Coquette
Tel. : +33 (0)1 47 95 60 00 • Fax : +33 (0)1 47 41 91 33
www.foodscience.bio-rad.fr

MANUFACTURER'S QUALITY CONTROL

All products manufactured and marketed by Bio-Rad are placed in a quality assurance system from receipt of the raw materials to marketing of the finished products.
Each batch of finished product undergoes quality control and it is marketed only if it is in compliance with the acceptance criteria.

The documentation relating to the production and control of each batch is preserved.

BIBLIOGRAPHIC REFERENCES

- **Balasubramanian D, K. Mathee (2009).** Comparative transcriptone analyses of *Pseudomonas aeruginosa*. Hum. Genomics, vol. 4. p. 349-361.

- **Husson MO, M. Hamze, S. Verhille, D. Izard. (2000).** *Pseudomonas* et *Burkholderia*. *In*: Précis de bactériologie Clinique. Freney F, F. Renaud, W. Hansen and C. Bollet. Eska. p.1259-1285.

- **Lisa A.T., P.R. Beassoni, M.J. Massimelli, L.H. Otero, C.E. Domenech (2007).** A glance on *Pseudomonas aeruginosa* phosphoryl-choline phosphatase, an enzyme whose synthesis depends on the presence of choline in its environment. Applied Microbiology. p. 255-262.

- **Lucchesi G.I., C. Pallotti, A. T. Lisa, C. E. Domenech (1998).** Constitutive choline transport in *Pseudomonas aeruginosa.* Microbiology Letters, vol. 162. p. 123-126 .

KEY WORDS

RAPID'*P.aeruginosa* / *Pseudomonas aeruginosa* / Water / Bottled water / Enumeration / Chromogen / Filtration / Medium.

Bio-Rad, 3 boulevard Raymond Poincaré • 92430 Marnes-la-Coquette
Tel. : +33 (0)1 47 95 60 00 • Fax : +33 (0)1 47 41 91 33
www.foodscience.bio-rad.fr