RAPID'*E.coli* 2 Supplement (For Water Testing) 355-5298

DEFINITION

RAPID'*E.coli* 2 for water testing allows the direct and simultaneous enumeration (without confirmation) of *Escherichia coli* and total Coliforms, by membrane filtration method.
It is particularly convenient for testing the water for human consumption and non-treated waters (such as waters from wells, lakes and rivers), which present an abundant interferant hydric flora, for which the standardised media are not suitable at all.

AFNOR CERTIFICATION according to AFNOR protocol Rev 1

The RAPID'*E.coli* 2 complete for water method is certified by AFNOR Certification as an alternative method to the reference standard EN ISO 9308-1, for the detection and enumeration of *Escherichia coli* and total Coliforms at 36°C **in waters with low level of suspended matters (among which network tap water and bottled water)** according to the reference for validation "Protocol for the Validation of a commercial method versus a reference method in the water microbiology field (revision 1)" (adopted by AFNOR Certification on 10/05/2010), under Attestation No: **BRD 07/20 - 03/11**.

Valid until: **03/2015**

BRD 07/20 - 03/11
Methods of water analysis
Certified analytical performances
www.afnor-validation.org

PRINCIPLE

The principle of the complete medium (supplemented RAPID'*E.coli* 2) relies on the simultaneous detection of 2 enzymatic activities: β-D-glucuronidase (GLUC) and β-D-galactosidase (GAL), by two chromogenic substrates:

- cleavage of the GAL specific substrate leads to form a precipitate giving a green coloration of the positive colonies for this enzyme (Coliforms),
- cleavage of the GLUC specific substrate leads to form a precipitate giving a pink coloration of the positive colonies for this enzyme (*E. coli*).

Coliforms (GAL+/GLUC-) form green colonies, *E. coli* (GAL+/GLUC+) form blue to violet colonies du to the superposition of both colorations.

The selective mixture in the supplement makes the medium inhibit the main interfering flora in water.

PRESENTATION

- **RAPID'*E.coli* 2 Supplement**
  Box of 6 vials **code 355-5298**
  (1 vial contains lyophilized in sufficient quantity for 100 ml of RAPID'*E.coli* 2 medium)

- **RAPID'*E.coli* 2 Kit for Water Testing**
  code 355-5296

Pack including:
- 6 vials x 100 ml RAPID'*E.coli* 2 medium
- 6 vials of supplement x 1 box
  (1 vial contains lyophilized in sufficient quantity for 100 ml of RAPID'*E.coli* 2 medium)

- **Complete RAPID'*E.coli* 2 Agar**
  55 mm x 20 dishes **code 356-3982**

STORAGE

- Lyophilized: +2-8°C in a dark place
- Expiration date and batch number are shown on the package.
- Re-hydrated vial: 7 days at 2-8°C in a dark place

THEORETICAL FORMULA
RAPID'*E.coli* 2 Supplement

Selective mix

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)

- **Sterile distilled water**
- **RAPID'*E.coli* 2 medium**
  100 ml x 6 bottles (code 355-5299)

See corresponding Technical Sheet

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EQUIPMENT REQUIRED (NOT SUPPLIED)
(non-exhaustive)

- Mixer-homogenizer
- Sterile pipettes (1 ml, 5 ml, etc)

RECONSTITUTION OF SUPPLEMENT RAPID'*E.coli* 2

Make up the lyophilisate by adding **1.1 ml of distilled water** to the bottle under aseptic conditions.
Shake until the lyophilisate is completely dissolved.

PREPARATION OF COMPLETE MEDIUM (RAPID'*E.coli* 2 + Supplement)

Under aseptic conditions add **1.0 ml** of rehydrated (or unfrozen) RAPID'*E.coli* 2 Supplement to 100 ml of sterile RAPID'*E.coli* 2 medium cooled and maintained at 47 ± 2°C (= complete medium).
NB: 0.1 ml remains in the vial.

Shake so as to homogenize thoroughly. Pour approximately 9 ml (8 to 10 ml) of the complete medium per sterile Petri dish (Ø = 55 mm). Leave to solidify on a cool, level surface.
1 vial of re-hydrated RAPID'*E.coli* 2 supplement allows to complete 100 ml of RAPID'*E.coli* 2 medium.

PROTOCOL

- **Preparation of samples**

According to the standard applicable to the product concerned.

- **Inoculation and incubation**

After filtration of 100 ml (or more, depending on the origin) of water to be tested, the membrane is deposited on the surface of the agar (square side upper most). Turn the dishes over and incubate **at the unique temperature of 36 ± 2°C for 21 hrs ± 3 hrs** (simultaneous enumeration of *E.coli* and total Coliforms).

READING AND INTERPRETATION

- **Colony count (UFC)**

After incubation, read the dishes membrane upside and proceed to count the characteristic colonies:
- Coliforms (other than *E. coli*) = GAL (+) colonies are green - *E. coli* = variable appearance:
  - dark blue to violet = GLUC (+) strain
  - grey-blue = GLUC weakly (+) strain
  - possible violet halo surrounded the typical colonies

As *Escherichia coli* belong to the Coliforms group, total Coliforms are enumerated by adding together blue and dark blue to violet colonies, and green colonies.

NB: Retain only dishes containing fewer than 50 characteristic colonies and fewer than 100 colonies in all.

- **Expression of results/Calculations**

Record the number of *E.coli* and/or Coliforms per unit volume of filtered water.

LIMITS OF USE

Certified quantitative microbiological reference materials (capsules, lenticules and pastilles) are commonly used in internal laboratory quality controls or inter-laboratory tests to check and validate the performances of the culture media.
Depending on the production method, some collection strains may present difficulties in growth due to physiological stress on the cells which, however, do not call into question the fertility performances of the culture medium used.
Some BioReference Pastilles (Pasteur Institute, France) containing *E. coli* and other Coliforms reference strains could therefore present weaker relative yields (RY < 66%) on the supplemented RAPID'*E.coli* 2 medium.

Therefore, to ensure the physiological integrity of cells, preparation of a fresh calibrated suspension from a strain cultured on a nutritive medium is recommended. The use of other commercialized reference materials remains possible.

PRECAUTIONS

- Avoid trapping any air bubbles underneath the membrane when depositing it on the agar. If necessary, gently and carefully flatten the membrane using tweezers.
- Comply with Good Laboratory Practice.

PERFORMANCES/QUALITY CONTROL OF THE TEST

The growth performances are verified with the following strains on complete supplemented RAPID'*E.coli* 2 medium:

STRAINS	Results after 18-24 hrs culture at 36°C	
	GROWTH	COLOR
*Escherichia coli** RIVM WR1**	+	Violet with violet halo
Aeromonas hydrophila	Inhibited	NA

**** Fertility yield: R = [66% - 150%]***
***** RIVM WR1 is equivalent to NCTC 13167***
NA: Not applicable

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QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product.
Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

KEY WORDS

RAPID'*E.coli* 2/Supplement/*Escherichia coli*/
Coliforms/Water/Enumeration/
β-D-glucuronidase/β-D-galactosidase/
Chromogenic/Filtration/Medium

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