RAPID'*E.coli 2*/Agar

355-5299
356-4024
355-5297

DEFINITION

A selective **chromogenic** medium used for direct enumeration **without confirmation of colonies** of *Escherichia coli* and other coliforms in food products for human and animal consumption (depth method) and in water (Membrane-filtration method).

Note:

In the context of NF VALIDATION (The Association Française de Normalisation) only enumerations of Escherichia coli and other coliforms in food products for human consumption are validated.

NF VALIDATION by AFNOR CERTIFICATION as per EN ISO 16140 protocol

The RAPID'*E.coli 2* method (**Enumeration of *E.coli* at 44°C**) has been certified NF Validation as a valid alternative method to the NF ISO 16649-2 standard for the enumeration of β-Glucuronidase-positive *Escherichia coli* at 44°C for **all food products intended for human consumption,** according to NF EN ISO 16140.

BRD 07/1 - 07/93
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
Certified by AFNOR Certification
www.afnor-validation.com

The RAPID'*E.coli* 2 method (**Enumeration of *E.coli* at 37°C**) has been certified NF Validation as a valid alternative method to the NF ISO 16649-2 standard for the enumeration of β-Glucuronidase-positive *Escherichia coli* at 37°C for **all food products intended for human consumption,** according to NF EN ISO 16140.

BRD 07/7 - 12/04
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
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The RAPID'*E.coli 2* method (**Enumeration of coliforms at 37°C**) has been certified NF Validation as a valid alternative method to the NF ISO 4832 standard for the enumeration of coliforms for **all food products intended for human consumption,** according to NF EN ISO 16140.

BRD 07/8 - 12/04
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
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- End of NF VALIDATION: please see the certificate BRD 07/1 - 07/93 (Enumeration of E.coli at 44°C), BRD 07/7 - 12/04 (Enumeration of E.coli at 37°C) and BRD 07/8 - 12/04 (Enumeration of coliforms at 37°C). These certificates are available from Bio-Rad representative or AFNOR Certification.

AOAC-RI VALIDATION

RAPID'*E.coli* 2 is validated by the AOAC Research Institute under the "Performance Methods Tested" status, under **Certificate n° 050601.**

NORDVAL VALIDATION

The RAPID'*E.coli 2* method (Enumeration of *E.coli* at 44°C) has NORDVAL approval as a valid alternative method to the NF ISO 16649-2 standard for the enumeration of β-Glucuronidase-positive *Escherichia coli* at 44°C for **all food products intended for human consumption,** according to ISO 16140.

The RAPID'*E.coli* 2 method (Enumeration of *E.coli* at 37°C) has NORDVAL approval as a valid alternative method to the NF ISO 16649-2 standard for the enumeration of β-Glucuronidase-positive *Escherichia coli* at 37°C

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for **all food products intended for human consumption,** according to ISO 16140.

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STANDARDS

- **FOOD MICROBIOLOGY**
- **NF ISO 16649-2 (July 2001)**:
  Food microbiology – Horizontal method for the enumeration of Glucuronidase-positive *Escherichia coli* β - Part 2: Technique of colony count at 44°C by means of 5-bromo-4-chloro-3-indolyl- β-D-glucuronate acid
  (IC: V08-031-2).

- **NF ISO 4832 (July 1991)**: Microbiology – General guidelines for the enumeration of coliforms - Colony count method.

PRINCIPLE

The principle of the medium relies on the simultaneous detection of 2 enzymatic activities:
β-D-Glucuronidase (GLUC) and
β-DGalactosidase (GAL). The medium contains 2 chromogenic substrates:

- one substrate specific to GAL that leads to blue coloration of colonies positive for this enzyme.
- one substrate specific to GLUC that leads to pink coloration of colonies positive for this enzyme.

E. coli (GAL +/GLUC +) form violet to pink colonies. Other coliforms (GAL +/GLUC-) form blue to green colonies.
Detection of GLUC makes the culture medium highly specific. *Escherichia coli* is in fact one of the only species of enterobacteria to possess this enzyme.

N.B.:

- *A few β-D-Glucuronidase-negative strains of E. coli exist, e.g. E. coli O157.*
- *Some serovars of Salmonella and a few species of Shigella possess the enzyme β-D-Glucuronidase (< 1.5%).*

PRESENTATION

- **Ready-to-use**
  100 ml x 6 bottles **code 355-5299**
  200 ml x 6 bottles **code 355-5297**

- **Dehydrated**
  500 g **code 356-4024**

STORAGE

- Ready-to-use: + 2° to 8°C in a dark place.
- Dehydrated: + 15° to 25°C, in carefully-sealed bottles in a cool, dry, dark place.
- Expiration date and batch number are shown on the package.
- Medium prepared by user from dry medium: 1 month at + 2° to 8°C in a dark place.

THEORETICAL FORMULA

Peptones	10 g
Sodium chloride	5 g
Yeast extract	3 g
Selective chromogenic mixture	6 g
Agar	13 g
Distilled water	1,000 ml

Final pH $_{(25°C)}$ = 7.2 ± 0.2

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)

- RAPID'*E.coli* 2 Supplement (Lyophilized): pack of 6 vials (1 vial contains lyophilized in quantity sufficient for 100 ml of RAPID'*E.coli 2* medium) (**code 355-5298**) (*utilization in water only*)
- Diluent(s)
- Distilled water

See corresponding Technical Sheet(s)

EQUIPMENT REQUIRED (NOT SUPPLIED)

(non-exhaustive)

- Scales
- Sterile weighing bags
- Grinder
- Mixer-homogenizer
- 125 ml bottles with autoclave-proof stoppers
- Sterile pipettes (1 ml, etc)
- Sterile Petri dishes (Ø = 55 mm and 90 mm)
- Water-bath precise to ± 1°C
- Thermostatically-controlled incubator or incubation room, precise to ± 1°C
- Autoclave
- All usual laboratory equipment

PREPARATION OF DEHYDRATED MEDIUM

Always shake before use
Dissolve 37 g of powder in 1 liter of distilled water. Wait for 5 minutes, then mix until a homogenous suspension is obtained. Heat gently, swirling frequently, then bring to the boil until completely dissolved. Dispense, then sterilize in autoclave at 121°C (± 1°C) for 15 minutes.
Reconstitution ratio: 37 g/l
500 g of powder reconstitutes 13.5 liters of medium.

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PROTOCOL

• Preparation of samples

According to the standard applicable to the product concerned.

• Inoculation

Food

- Using sterile pipettes, transfer 1 ml of sample to be tested (liquid product) or 1 ml of stock suspension (other products) and/or 1 ml of its decimal dilutions in sterile Petri dishes (Ø= 90 mm).
- Rapidly pour about 15 ml of melted medium, cooled to 44-47°C.
- Homogenize.
- Leave to solidify on a cool, level surface.

Water

- See corresponding Technical Sheet.

• Incubation

- Turn the dishes over and incubate at:
  - 37°C ± 1°C for 21 hours (± 3 h) for simultaneous enumeration of *E.coli* and coliforms (= total coliforms)*.
  - 44°C ± 1°C for 21 hours (± 3 h) for enumeration of *E. coli**.

* Incubation temperature for which RAPID'*E. coli* 2 method has been certified in the context of NF VALIDATION mark.

READING AND INTERPRETATION (food products)

For water, refer to the Technical Sheet for Water Testing application.

• Colony count (CFU)

- After incubation, count the characteristic colonies:
- Coliforms (other than *E.coli*) = blue
- *E. coli* = violet to pink.

As *Escherichia coli* is a species belonging to the coliform group, total coliforms are enumerated by adding together blue-green colonies and violet to pink colonies.

N.B.:

- Select only dishes containing fewer than 150 characteristic colonies and fewer than 300 colonies in all.

- Depending on the various calculation methods, dishes containing no colonies can be selected.

• Expression of results/Calculations

Refer to standard NF ISO 7218 for the calculation method.

PRECAUTIONS

- For ready-to-use media prepared in advance, avoid any prolonged overheating during fusion (in general, 20 minutes is enough to obtain a homogenous liquid agar) and supercooling (maximum 6h). To conserve an optimal quality the medium must not undergo more than 2 regeneration cycles (maximum total 6h).
- The medium may look frothy after gelification in bottles. It nevertheless conserves all its qualities when the froth disappears after melting and shaking.
- As development of colonies at the bottom of the Petri dish may interfere with reading, the period between the deposition of the inoculum at the bottom of the dish and the dispensing of the culture medium be limited.

It is preferable to use a double-layered medium at 37°C for the enumeration of *E. coli* and coliforms in matrices containing abundant mesophilic flora. The aim of the second layer is to limit invasion of the surface, which can interfere with reading (untreated milk, raw meat).

- 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.

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.

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PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains.

STRAIN	Appearence of colonies after 18-24 h culture at 44°C	
	GROWTH	COLOR
Escherichia coli WDCM 00090	+	Violet

STRAINS	Aspect of colonies after 18-24 h culture at 37°C	
	GROWTH	COLOR
Klebsiella oxytoca SDP 12.1.1	+	Blue
Proteus mirabilis ATCC 25933	+	Non typical
Staphylococcus aureus WDCM 00034	-	NA

NA: not-applicable

KEY WORDS

RAPID'*E.coli* 2/*Escherichia coli*/Coliforms/Food products/Water/Detection/Enumeration/Bêta-DGlucuronidase/Bêta-D-Galactosidase/ Chromogen/Filtration/Medium.

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