RAPID'*E.coli* O157:H7/Agar

356-4748

FIELD OF APPLICATION

Selective **chromogenic** medium for the detection, isolation and presumptive identification of *Escherichia coli* O157:H7 in products for use in human food and environmental samples.

PRINCIPLES

The RAPID'*E.coli* O157:H7 medium is a selective medium combining chromogenic substrates and biochemical indicators. This combination provides direct presumptive identification of *E.coli* O157:H7, including atypical strains, among the interfering flora on the basis of the specific metabolic and enzymatic profiles observed.

The selectivity of the medium is increased by adding selective agents: novobiocin (10 mg/l) and potassium tellurite (0.8 mg/l).

NF VALIDATION by AFNOR CERTIFICATION as per EN ISO 16140 protocol

The RAPID'*E. coli* O157:H7 method has been certified NF VALIDATION as an alternative to the reference standard, NF EN ISO 16654, according to the ISO 16140 protocol, for the detection of *Escherichia coli* O157:H7 **for all human food products** and **for environmental samples.**

BRD 07/14 - 09/07
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
Certified by AFNOR Certification
www.afnor-validation.com

For information on expiry of the NF validation, see the certificate BRD 07/14 - 09/07 available from Bio-Rad customer service or AFNOR Certification.

AOAC-RI VALIDATION

RAPID'*E.coli* O157:H7 has been validated by the AOAC Research Institute according to the "Performance Methods Tested" protocol, under **attestation number: 060701.**

STANDARD REFERENCES

NF EN ISO 16654 (July 2001)
Food microbiology – Horizontal method for the detection of *Escherichia coli* O157

PRESENTATION

Dehydrated

- 100g **code 356-4748**
- Novobiocin (one 1-g bottle) **code 356-4610**

PRESERVATION/VALIDITY/LOT

- Dehydrated: 2 - 8°C, Carefully closed bottle
- Expiry date and lot number are indicated on the packaging.

THEORETICAL FORMULA

Enrichment mixture	58 g
Selective agents	6.25 g
Chromogenic mixture	0.75 g
Agar	15 g
Distilled water qsp	1,000 mL

final pH (25°C) = 6.9 +/- 0.2

OTHER PRODUCT(S) REQUIRED NOT SUPPLIED

- mTSB broth:

Ready to use (with novobiocin):
6 x 225-ml bottles **(ex. code 355-5426)**
Dehydrated (base): 500 g **(ex. code 356-4426)**

- Novobiocin (ex. One 1-g bottle, **code 356-4610**)
- Potassium tellurite

See the corresponding Technical Data Sheet(s)

EQUIPMENT REQUIRED, NOT SUPPLIED

(non-exhaustive list)

- Scales
- Stomacher bags with filter
- Grinder
- Stirrer-homogenizer
- Sterile Petri dishes (Ø = 90 mm)
- Sterile pipettes (1 mL)
- Filter cones for micropipettes (100(0)µL)
- Inoculating loops
- Sterile Pasteur Pipettes
- Water bath at ± 1 °C
- Oven or thermostated unit at ± 1 °C precision
- Immunoconcentration beads for *E. coli* O157 and magnetic rack

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RAPID'*E.coli* O157:H7/Agar

- Latex confirmation tests for *E.coli* O157:H7
- Non-selective agar or broth (Columbia blood agar (ex. code 356 3784), TCS broth (ex. code 355-3455)
- All common laboratory equipment

PREPARATION OF THE DEHYDRATED MEDIUM

"Always shake before each use"

Dissolve 80 g of powder in 1 liter of distilled water. Mix to obtain a homogenous suspension. Adjust the pH to 6.9 +/-0.2 at 25℃ using either NaOH or HCl 1N. Bring to a boil until complete dissolution. Avoid overheating. DO NOT AUTOCLAVE. Cool to 45-50℃ in the water bath. Sterilely add the novobiocin (code 356-4610) (qsp 10 mg/L in the complete medium) and the potassium tellurite (qsp 0.8 mg/L in the complete medium).
Mix and distribute in the sterile Petri dishes. Let dry overnight at room temperature.
Final pH: 6.9 +/- 0.2 at 25℃

Novobiocin supplement: dissolve 100 mg of novobiocin (code 356-4610) in 1 ml of sterile distilled water. Sterilize by filtering through a 0.2-µm absolute filter and using a disposable syringe. This novobiocin solution can be stored for 1 month at 4℃ in an opaque bottle.

Reconstitution rate: 80 g/L
100 g of powder can be used to make 1.25 liters of medium

PROTOCOLS

Detection of *Escherichia coli* O157:H7 in ηg or ηml of sample:

Note: Certain precautions for use are associated with the application of these methods and are given on page 3.

STANDARDIZED METHOD:

- **Preparation of the samples / Enrichment**

To be performed according to the standards of the product in question.

- **Immunoseparation**

Strictly follow the supplier's recommendations for the immunoseparation protocol.

- **Isolation and Incubation**

Take 50 µl of beads washed and placed in suspension after immunoseparation with a sterile micropipette and perform isolation following the conventional techniques on RAPID'*E.coli* O157:H7 agar, in parallel with a dish of CT-SMAC. Incubate at 37℃ ± 1℃.

ALTERNATIVE METHOD:

- **Preparation of the sample / Selective enrichment**

- Culture η g or η ml of sample in 9 x η ml of mTSB broth + novobiocin (20 mg/l).
- Incubate at 41.5 ℃ for 16-24 hours.

- **Immunoseparation**

- Use a system of paramagnetic beads coated with specific antibodies for capturing *E.coli* O157. Carefully follow the supplier's recommendations for the immunoseparation protocol.

- **Isolation and Incubation**

- Take 50 µl of beads washed and placed in suspension after immunoseparation with a micropipette with sterile cones and perform isolation in streaks following the conventional techniques on RAPID'*E. coli* O157:H7 agar. Incubate for 24h ± 2h at 37℃ ± 1℃.

Note: In the context of NF VALIDATION mark, no samples of over 25 g were tested.

READING AND CONFIRMATION

• Reading

Read after incubation for 24h ± 2h.
Typical *Escherichia coli* O157:H7 (sorbitol – and β–glucuronidase –) present characteristic bright, bulging colonies measuring 1 to 2 mm, dark blue to black in color with a slight black precipitate around the edges of the colony.
Atypical β–glucuronidase + *Escherichia coli* O157:H7 form colonies of the same type.
Strains of atypical sorbitol + *Escherichia coli* O157:H7 are also detected. These colonies will have a blue to turquoise color with a weak black precipitate around the edges of the colony.

Note: The agar will turn completely red in the presence of pure strains of *E.coli* O157:H7.
On agar with a mixture of strains, typical *Escherichia coli* O157:H7 give dark blue to black colonies with a slight black precipitate around the edges of the colony, sometimes combined with a red halo.

• Confirmation of characteristic colonies

In the context of NF VALIDATION Mark, positive results must be confirmed in one of the following way:

1-Using the **conventional tests** described in the standardised CEN or ISO reference methods (with purification step) or using **validated nucleic probes**, for example: **iQ-Check™** *E.coli* O157:H7 PCR method (code **357-8114)** using isolated colonies (with or without purification step).

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2- Using of **latex** tests for O157 and H7 starting with 1 to 3 isolated colonies. An isolation step must be performed in case of confirmation with 2 latex tests.

Note:
- When using the latex tests, carefully follow the manufacturer's instructions and recommendations for use.
- For characteristic colonies giving a positive O157 latex test and a negative H7 latex test, the laboratory will have to apply the appropriate measures to ensure the validity of the results obtained.

3- The use of any **other NF VALIDATION certified method** with a **different principle** than that of RAPID'*E.coli* O157:H7. The validated protocol for this method will have to be followed in whole, except for cases with preliminary steps common to both methods.

- In case of discordant results (positive with the alternative method, not confirmed with the tests described above, and especially for Latex tests), the laboratory will have to apply the appropriate measures to ensure the validity of the results obtained.

PRECAUTIONS FOR USE

- **Performing the immunoseparation step** requires sufficient training and regular practice of the technique. Following these precautions for use is a prerequisite for obtaining valid, reliable results**.**
- When applying immunoseparation, viscous or fatty samples may cause interference in the magnetic bead capture (low retrieval, reduced specificity of the antibody action). See the suppliers' technical solutions for handling such samples.
- **Reading the latex tests** may require prior training, notably for interpreting the agglutination of the H7 flagellar antigen, which can be very fine.
- The precautions for use relative to the handling of potentially contaminated products in a microbiology laboratory must be followed.
- Before using the boxes of RAPID'*E.coli* O157:H7, let them dry, in compliance with the EN ISO 11133-1 standard, at 25℃-50℃ until the droplets disappear from the surface of the medium. Avoid prolonged drying, however, as this could alter the performances of the medium.
- Follow Good Laboratory Practices (EN ISO 7218)

QUALITY CONTROL

All products manufactured and sold by Bio-Rad are placed under a quality assurance system from reception of the raw materials to the sale of the finished products.
Each lot of finished products undergoes quality control and cannot be sold unless it complies with the acceptance criteria.
Documentation relative to the production and inspection of each lot is archived.

QUALITY AND PERFORMANCES OF THE TEST

MICRO-ORGANISMS	Appearance of the colonies after 24 hours at 37 ℃	
	GROWTH	COLOR
Escherichia coli O157:H7 ATCC 700728	+	Blue to black colony with black precipitate around the edges
Escherichia coli O157:H7 ATCC 43888	+	Blue to black colony with black precipitate around the edges
Escherichia coli ATCC 25922	partial to inhibition	Green colony with green precipitate around the edges
Staphylococcus aureus ATCC 6538	-	NA

KEY WORDS

RAPID'*E. coli* O157:H7 / *Escherichia coli* / *Escherichia coli* O157 / *Escherichia coli* O157:H7 / Detection / Food Products / Environment / Chromogenic / Medium.

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