RAPID’ E. coli O157:H7

Catalog# Description
Dehydrated base
3564748 100 g
3564610 Novobiocin (one 1 g bottle)

FIELD OF APPLICATION
RAPID’ E. coli O157:H7 is a 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.

PRINCIPLE
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).

VALIDATIONS

<table>
<thead>
<tr>
<th>Scope</th>
<th>Reference method</th>
<th>Target</th>
<th>Certificate reference</th>
<th>Validation protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat products</td>
<td>NF EN ISO 16654</td>
<td>Escherichia coli O157:H7</td>
<td>BRD 07/14-09/07</td>
<td>EN ISO 16140-2</td>
</tr>
<tr>
<td>Dairy products</td>
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<td>Fruits and vegetables</td>
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<td>Composite foods</td>
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</thead>
<tbody>
<tr>
<td>Raw ground beef</td>
<td>BAM 8th Edition; Chapter 4A. Microbiology Laboratory</td>
<td>Escherichia coli O157:H7</td>
<td>060701</td>
<td>AOAC-RI</td>
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<tr>
<td>Fresh spinach</td>
<td>Guidebook, Chapter 5C.00</td>
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STANDARDS
- NF EN ISO 16654 (July 2001) - Microbiology of food and animal feeding stuffs - Horizontal method for the detection of Escherichia coli O157 Modified by Amendment 1 : annex B : result of interlaboratory studies (June 2017).
- US FDA Bacteriological Analytical Manual Chapter 4A – Diarrheagenic Escherichia coli
- USDA Microbiology Laboratory Guidebook Chapter 5C.00 – Detection, Isolation and Identification of Top Seven Shiga Toxin-Producing Escherichia coli (STECs) from Meat Products and Carcass and Environmental Spongess
STORAGE / SHELF-LIFE / BATCH
- Dehydrated: +2° to 8°C, in carefully sealed package.
- Plates prepared from dehydrated: 2 weeks at +2-8°C, in carefully sealed package, in a dry and dark place.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Enrichment mixture</td>
<td>58 g</td>
</tr>
<tr>
<td>Selective agents</td>
<td>6.25 g</td>
</tr>
<tr>
<td>Chromogenic mixture</td>
<td>0.75 g</td>
</tr>
<tr>
<td>Agar</td>
<td>15 g</td>
</tr>
<tr>
<td>Distilled water qsp</td>
<td>1,000 ml</td>
</tr>
</tbody>
</table>

Final pH (25°C) = 6.9 ± 0.2

PREPARATION COMPLETE MEDIUM
- **Preparation of medium**
  - **Always shake before use**
    - Dissolve 80.0 g of powder in 1 L of distilled water.
    - Mix to obtain a homogenous suspension.
    - Bring to a boil until complete dissolution. Avoid overheating.
    - DO NOT AUTOCLAVE.
    - Cool to 45-50°C in a water bath.
    - Using aseptic technique, add the novobiocin (3564610) (qsp 10 mg/l in the complete medium) and the potassium tellurite (qsp 0.8 mg/l in the complete medium).
    - Mix thoroughly.
    - Pour into Petri dishes (thickness ~ 4 mm)
    - Let dry overnight at room temperature.
    - The final pH to 6.9 +/-0.2 at 25°C.

- **Preparation of 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 2-8°C in an opaque bottle.

Reconstitution ratio: 80.0 g/l
100 g of powder makes 1.25 L of base medium.

PRODUCTS AND MATERIALS REQUIRED (Not supplied and non-exhaustive list)

**Equipment**
- Scales
- Sterile weighing bags
- Stirrer-homogenizer
- Sterile Petri dishes (Ø = 90 mm)
- Sterile pipettes (1 mL)
- Filter cones for micropipettes
- Inoculating loops
- Sterile Pasteur Pipettes
- Water bath at ± 1 °C
- Oven or thermostated unit at ± 1 °C
- Latex confirmation tests for *E. coli* O157:H7
- All common laboratory equipment

**Reagent required for the preparation of sample**
- mTSB broth Ready to use (with novobiocin) 6 x 225 ml bottles (ex.3555426), dehydrated (base) 500g (ex. 3564426)
- Novobiocin one 1 g bottle (ex. 3564610)

**For immunoseparation**
- Immunoconcentration beads for *E. coli* O157 and magnetic rack

**For confirmation**
- Latex confirmation tests for *E. coli* O157:H7
- iQ-Check *E. coli* O157:H7 Real-Time PCRKit: 3578114

**PROTOCOL**

Detection of *Escherichia coli* O157:H7 in *n* g or *n* ml of sample:

**STANDARDIZED METHOD:**
- **Preparation of sample**
  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, repeat the inoculation on CT-SMAC agar.
  - Incubate at 37 °C ± 1 °C.

**ALTERNATIVE METHOD:**

- **Preparation of the sample /Selective enrichment**
  - Culture η g or η ml of sample in 9 x η ml of mTSB broth preheated at 41.5 °C + novobiocin (20 mg/l).
  - Incubate at 41.5 ± 1 °C for 16-24 hr.

- **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 24 ± 2 hr at 37 ± 1 °C.

**READING AND CONFIRMATION**

- **Reading**

  Read after incubation for 24 ± 2 hr.

  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.

- **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 CEN or ISO standard methods (including the purification step).
  2- Using nucleic probes as described in the ISO 7218 standard (for example, iQ-Check *E. coli* O157:H7 real-time PCR kit, catalog# 3578114) using isolated colonies (with or without purification step).
  3- Using latex tests for O157 and H7 starting with 1 to 3 isolated colonies. An isolation step must be performed in case of confirmation with two latex tests.
  4- Using any other NF VALIDATION certified method based on a different principle from that of RAPID’E.coli O157:H7. The validated protocol of the second method must be respected in its entirety. All steps preceding the detection step used as a starting point for confirmation must be 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**

- Follow Good Laboratory Practices (EN ISO 7218).
- The precautions for use relative to the handling of potentially contaminated products in a microbiology laboratory must be followed.
- In the context of NF VALIDATION mark, no samples of over 25 g were tested.
- Before using the boxes of RAPID’E.coli O157:H7, let them dry, in compliance with the EN ISO 11133 standard, at 25°C-50°C until the droplets disappear from the surface of the medium. Avoid prolonged drying, however, as this could alter the performances of the medium.
- 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.
• The agar will turn completely red in the presence of pure strains of \textit{E. coli} O157:H7.
• On agar with a mixture of strains, typical \textit{E. 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.
• When using the latex tests, carefully follow the manufacturer’s instructions and recommendations for use.
• Reading the latex tests may require prior training, notably for interpreting the agglutination of the H7 flagellar antigen, which can be very fine.
• 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.

TECHNICAL SUPPORT IN THE UNITED STATES
In the United States, for technical assistance please call (800) 4BIORAD. Select option 2 for technical support. To place an order, please call (800) 4BIORAD and press option 1 for customer care.

QUALITY CONTROL
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.

QUALITY AND PERFORMANCE OF THE TEST
See quality certificate available on [www.bio-rad.com/certificate](http://www.bio-rad.com/certificate) (Catalog#/ref# and Lot# number are required)

KEY WORDS

For more information about Bio-Rad Food Testing products, visit our website: [www.bio-rad.com/foodscience](http://www.bio-rad.com/foodscience)