

mTSB/Broth (modified Tryptone Soy Broth)

355-5426
356-4426

DEFINITION

Selective enrichment broth for *Escherichia coli* O157:H7 in products intended for human and animal consumption.

STANDARDS

FOOD MICROBIOLOGY

- **NF EN ISO 16654 (July 2001):** Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Escherichia coli* O157.

PRINCIPLE

The standard Tryptone Soya Broth has been modified by increasing the buffer properties for a good recovering and growth of *Escherichia coli* O157, while addition of bile salts N°3 inhibits the growth of Gram positive bacteria. Novobiocin is used for the inhibition of *Proteus* spp and some others Gram negative bacteria. Tryptone, soy peptone and dextrose are respectively nitrogen and carbon energy sources that facilitates bacterial growth. Sodium chloride maintains osmotic balance, while dipotassium phosphate acts as buffering agent.

PRESENTATION

- **Ready to use (mTSB + Novobiocin)**
225 ml x 6 bottles **code 355-5426**
- **Dehydrated (without Novobiocin)**
500 g **code 356-4426**
- **Novobiocin Supplement**
1g **code 356-4610**

STORAGE

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

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)

- Distilled water
- IMS kits

THEORETICAL FORMULA

Dehydrated

Tryptone	17 g
Sodium chloride	5 g
K ₂ HPO ₄	4 g
Dextrose	2.5 g
Soy peptone	3 g
Bile Salts N°3	1.5 g
Distilled water	1,000 ml
Final pH _(25°C) = 7.2 ± 0.2	

Ready to use with novobiocin

Tryptone	17 g
Sodium chloride	5 g
K ₂ HPO ₄	4 g
Dextrose	2.5 g
Soy peptone	3 g
Bile Salts N°3	1.5 g
Novobiocin	20 mg
Distilled water	1,000 ml
Final pH _(25°C) = 7.2 ± 0.2	

EQUIPMENT REQUIRED (NOT SUPPLIED) (non-exhaustive)

- Scales
- Sterile weighing bags
- Grinder
- Mixer-homogenizer
- Sterile bottle (300 ml)
- Sterile pipettes (1 ml, etc)
- Sterile spreaders
- Sterile membrane filters (0.2 µm)
- Water-bath precise to ± 1°C
- Thermostatically-controlled incubator or incubation room, precise to ± 1°C
- Autoclave
- IMS equipment
- All usual laboratory equipment

PREPARATION OF DEHYDRATED MEDIUM

Always shake well before use.

Weigh 33 grams of powder and add 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and autoclave at 121°C for 15 minutes. Cool to 47°C and add aseptically 0.2 ml of novobiocine supplement (in order to have 20 mg/l of novobiocine in the complete medium). Mix well and distribute aseptically into sterile containers.

Reconstitution ratio: 33 g/l
500 g of powder makes 15.1 liters of medium.

Novobiocine supplement (code 356-4610)

Dissolve 100 mg of novobiocine in 1 ml of deionised water. Sterilize by filtration using a 0.2 µm absolute filter and a single-use syringe. This solution of novobiocine could be kept for several months at 4°C in a brown bottle.

PROTOCOL

Preparation of samples

Add a test portion of 25g or 25 ml to 225 ml of mTSB with novobiocin.

Standard method

• Incubation

Incubate the initial suspension at 41.5°C for 6h and subsequently for a further 12 to 18 h (i.e. a total elapsed time of 18 h to 24 h).

A 6 h incubation followed by immunomagnetic separation and plating onto selective agars can yield a presumptive positive result which can become negative after a further 18h incubation.

• Immunomagnetic separation (IMS)

IMS should be carried out after 6h and again, if necessary after 12 h to 18 h of incubation.

The manufacturer's instruction should be followed concerning the procedure and method for the use of immunocapture kits and the equipment needed.

• Plating out

Using a mechanical-type pipettor, transfer 50 µl of the washed and re-suspended magnetic particles to a pre-dried plate of cefixime tellurite sorbitol MacConkey, and also 50 µl to a pre-dried plate of the second isolation medium.

Incubate the CT-SMAC at 37°C for 18 h to 24h and incubate the second selective agar at its recommended temperature and specified incubation time.

Alternative methods

The mTSB with novobiocin is also used in two alternative methods:

- **RAPID[®] E. coli O157:H7 Agar**
500 g **code 356-4747**
- **iQ-Check[™] E. coli O157:H7 Kit** **code 357-8114**
1 kit

For detailed protocol, please refer you to the corresponding technical sheets of these methods.

PRECAUTIONS

Comply with Good Laboratory Practice.

PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with specific strains. Please refer to the quality control certificate of each product.

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

mTSB / *Escherichia coli* O157 / Food products / Detection.