



# TRYPTIC SOY BROTH MODIFIED (mTSB) NOVOBIOCIN ANTIMICROBIC SUPPLEMENT

Dehydrated culture medium and supplement, ready-to-use flasks

## 1 - INTENDED USE

With the addition of novobiocin, Tryptic Soy Broth Modified (mTSB) is used for the selective enrichment of *Escherichia coli* O157 in foods.

## 2 - COMPOSITION \*

TRYPTIC SOY BROTH MODIFIED (MTSB), DEHYDRATED MEDIUM	
TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER	
Pancreatic digest of casein	17.0 g
Soy peptone	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	4.0 g
Glucose	2.5 g
Bile Salts N° 3	1.5 g

### NOVOBIOCIN ANTIMICROBIC SUPPLEMENT - VIAL CONTENTS

Novobiocin	10 mg
------------	-------

### TSB MODIFIED, READY-TO-USE FLASKS – TYPICAL FORMULA PER LITRE

Tryptic Soy Broth Modified (mTSB)	33 g
Novobiocin	20 mg
Purified water	1000 mL

\*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

## 3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

mTSB was devised by Doyle and Shoeni<sup>1</sup> by increasing the concentration of the phosphate buffer of Tryptic Soy Broth and adding bile salts n° 3 and novobiocin with the aim of developing a procedure that could specifically isolate *E. coli* O157:H7 from foods.

The medium is recommended by ISO 16654<sup>2</sup> for the preparation of the initial suspension of the sample and for the enrichment procedure for the detection of *E. coli* O157 in foodstuffs. It corresponds to medium n° 156 of FDA BAM.<sup>3</sup>

Pancreatic digest of casein and soy peptone provide nitrogen, carbon, and trace elements for microbial growth. Dipotassium hydrogen phosphate is used as buffering agent to control the pH in the medium. Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium. Glucose is a source of carbon and energy. Selectivity is provided by the presence of bile salts n° 3 and novobiocin with a marked antibacterial activity against Gram-positive bacteria.

### 4A- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 33 g in 1000 mL of cold purified water. Mix well and, if necessary, heat slightly to completely dissolve the powder. Distribute 225 mL into flasks of suitable capacity and sterilise by autoclaving at 121°C for 15 minutes. Cool to room temperature and to each 225 mL flask add, under aseptic conditions, 2.25 mL of Novobiocin Antimicrobial Supplement (REF 4240045), reconstituted with 5 mL of sterile purified water. Final concentrations: 4.5 mg/225 mL or 20 mg/litre. The remaining novobiocin solution can be stored at 2-8 °C for one month.<sup>5</sup>

### 4B- DIRECTIONS FOR READY-TO-USE FLASKS

Bottled liquid medium is ready to use.

## 5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared flasks appearance	yellow, limpid
Freeze-dried selective supplement	short, dense, white pellet; colourless clear solution after reconstitution
Final pH of complete medium (at 20-25°C)	7.4 ± 0.2

## 6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Tryptic Soy Broth Modified (mTSB)	Dehydrated medium	402155M2	500 g (15.1 L)
Novobiocin Antimicrobial Supplement	Freeze-dried supplement	4240045	10 vials (10 mg/vial)
TSB Modified	Ready-to-use flasks	512155M3	6 x 225 mL

## 7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops, pipettes and Petri dishes, incubator and laboratory equipment as required, flasks, Erlenmeyer flasks, ancillary culture media and reagents.

## 8 - SPECIMENS

Foods, animal deeding stuffs, food chain samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.<sup>2</sup>

## 9 - TEST PROCEDURE

- Prepare the initial suspension by adding the test sample portion to Tryptic Soy Broth Modified with novobiocin (mTSB-N), prewarmed to 41.5 °C to obtain a ratio of 1/10 (e.g., 25 g of sample + 225 mL of mTSB-N)
- Incubate at 41.5°C ± 1°C for 6 h and subsequently for a further 12 to 18 h. The use of homogenisation bags with a filter strip is recommended.





- E. coli* O157 cells are separated and concentrated using immunomagnetic beads coated with antibodies to *E. coli* O157 after 6 h and again, if necessary, after a further 12 to 18 h incubation.
- 50 µl of immunomagnetic concentrated broth are sub-cultured onto Mac Conkey Sorbitol Agar with CT Supplement (CT-SMAC) and onto a second selective isolation agar of laboratory choice (e.g. Mac Conkey Sorbitol MUG Agar REF 401669 or Chromogenic *E. coli* O157 Agar REF 405581). CT-SMAC is incubated at  $37 \pm 1^\circ\text{C}$  for 18 to 24 h. The second agar of choice should be incubated following the IFU's recommended procedures.

### 10 - READING AND INTERPRETATION

The presence of microorganisms is indicated by a varying degree of turbidity, specks and flocculation in the liquid medium.

### 11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T° / T / ATM	EXPECTED RESULTS
<i>E. coli</i> O157 ATCC 43894	41.5°C / 18-24 H / A	growth
<i>S. aureus</i> ATCC 25923	41.5°C / 18-24 H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection; NCTC: National Collection of Type Cultures

### 12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale representative samples of all lots of dehydrated and ready-to-use Tryptic Soy Broth Modified (mTSB) supplemented with Novobiocin Antimicrobial Supplement (REF 4240045) are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.

Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of target organisms in test tubes, incubating at 41.5°C for 18-24 hours and recording the highest dilution showing growth in Reference Batch ( $G_{\text{RB}}$ ) and in Test Batch ( $G_{\text{TB}}$ ). Productivity is tested with the following target strains: *E. coli* O157 ATCC 43888, *E. coli* O157 ATCC 43894. The productivity index  $G_{\text{RB}}/G_{\text{TB}}$  for each test strain shall be  $\leq 1$ . Productivity and selectivity are tested together by inoculating approximately 100 CFU of the target strain *E. coli* O157 NCTC12900 and 1000 CFU of the non-target strain *S. aureus* ATCC 25923 into the same tube and incubating at 41.5 ± 1°C for 6 hours. After incubation of the inoculated tubes and subculture on MacConkey Sorbitol plates, the target strain will show more than 10 colonies per plate.<sup>2</sup>

Selectivity is assessed by dilution to extinction method with following non-target strains: *E. coli* ATCC 25922, *K. pneumoniae* ATCC 27736, *E. faecalis* ATCC 29212 and *S. aureus* ATCC 25923. After incubation at 41°C for full 24 hours the growth of Gram- positive strains is totally inhibited while the growth of Gram-negative non-target strains is partially or not inhibited.

### 13 - LIMITATIONS OF THE METHOD

- No standard methods are available for all pathogenic *E. coli* and the existing methods are either adapted from general methods for isolating *E. coli* or are developed for a specific group of pathogenic *E. coli*. Regardless of methods, however, it is important that isolates are identified biochemically as *E. coli*, in addition to testing for virulence factors associated with the respective pathogenic group.<sup>4</sup>

### 14 - PRECAUTIONS AND WARNINGS

- The medium base, the supplement and the ready-to-use flasks are for microbiological control and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplement shall be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media must be handled with suitable protection. Novobiocin Antimicrobial Supplement is classified as hazardous. Before use, consult the Safety Data Sheets.
- This culture medium contains raw materials of animal origin. The ante and post mortem controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website [www.biolifeitaliana.it](http://www.biolifeitaliana.it), describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Be careful when opening screw cap flasks to prevent injury due to breakage of glass. Be careful when opening the metal ring of the supplement vial to avoid injury.
- Ready-to-use flasks are subject to terminal sterilization by autoclaving and by aseptic addition of novobiocin.
- The supplement is sterilized by membrane filtration.
- Each flask of this culture medium is for single use only.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplement as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheets are available on the website [www.biolifeitaliana.it](http://www.biolifeitaliana.it).
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

### 15 - STORAGE CONDITIONS AND SHELF LIFE





### Ready-to-use medium in flasks

Upon receipt, store flasks in their original pack at +2/+8°C away from direct light. If properly stored, the flasks may be used up to the expiration date. Do not use the flasks beyond this date. Flasks from opened secondary packages can be used up to the expiration date. Opened flasks must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use flasks with signs of deterioration (e.g., microbial contamination, abnormal turbidity, precipitate, atypical colour).

### Dehydrated medium

Upon receipt, store at +10/+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

### Freeze-dried supplement

Upon receipt, store the product in the original package at +2/+8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type and the applied storage conditions (temperature and packaging). According to ISO 21567 the medium base and the novobiocin solution may be stored for up to one month at +2°C/+8°C.<sup>5</sup> At the time of use novobiocin should be added to the medium base.

### 16 - REFERENCES

1. Doyle MP, Schoeni JL. Isolation of Escherichia coli O157:H7 from retail fresh meats and poultry. *App Environ Microbiol* 1987; 53:2394-96.
2. ISO 16654:2001/Amd 2:2023. Microbiology of food and animal feeding stuffs- Horizontal method for detection of E.coli O157-Inclusion of performance testing of all culture media and reagent
3. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), online. BAM Media M156: Trypticase Soy Broth Modified (mTSB). Content current as of: 12/06/2017
4. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.
5. ISO 21567:2004 Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Shigella spp.

### TABLE OF APPLICABLE SYMBOLS

or Catalogue number	Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

### REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2022/11
Revision 3	Update of chapter 12 according to ISO 16654:2001/Amd 2:2023	09/2025

Note: minor typographical, grammatical, and formatting changes are not included in the revision history

