Product :
TRYPTIC SOY BROTH MODIFIED

Also known as
TSBm

Specification
Liquid culture medium used for the selective enrichment of Enterohaemorrhagic *Escherichia coli* (EHEC) in food, according to the ISO Standard 16654:2001.

Formula * in g/L
Tryptone 17,00
Soy peptone..... 3,00
Dextrose..... 2,50
Bile salts No.3..... 1,50
Sodium chloride..... 5,00
Bi-potassium phosphate.....4,00

Final pH 7.4 ±0.2 at 25 ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions
Dissolve 33 g in 1 L of distilled water. Distribute in volumes of 500 mL/container and sterilize in the autoclave at 121ºC for 15 minutes. Cool to 50ºC and aseptically add a vial of Novobiocin Selective Supplement (Art. No. 06-139LYO1) to each 500 mL of sterile medium. Homogenize and distribute into the final containers.

Description
The actual formulation of the medium complies with the European Norm EN-ISO 16654:2001, horizontal method for the detection of *Escherichia coli* O157.
The modification of the classical Tryptic Soy Broth was proposed in 1987 by Doyle and Schoenli by adding 1,5 g/L of bile salts No.3 to eliminate the non-enteric bacteria and the selectivity is reinforced by the addition of an antibiotic to inhibit the growth of Gram positive microbiota.
A broth with the same composition but with a higher (1,5%) concentration of salt and lesser (0,1 mg/L) novobiocin was used in the isolation and culture of *Shigella* from food samples.
The European methodology (EN, ISO, UNE, CCFRA, DIN, etc.) uses novobiocin at 20 mg/L concentration, but American methods (FDA, BAM, AOAC) pefers a mixture of antibiotics: cefixime 0,05 mg/L to suppress the growth of *Proteus spp.*; cefsulodine 10 mg/L to inhibit *Aeromonas* and *Pseudomonas* and vancomycin 8 mg/L to eliminate Gram positive bacteria. Such medium was proposed in 1995 by Weagant and collaborators. It is also known as "EHEC Broth" or EEE Broth".

Technique

The European Methodology proposes four steps in the Escherichia coli O157 detection:

1. Enrichment of the sample by incubation at 41ºC ± 0.5 ºC in TSBm with antibiotic(s) for 6 hours followed by a supplementary period of 12-18 hours.
2. Separation and Concentration with immunomagnetic particles.
3. Isolation and presumptive identification from the immunomagnetic particles onto selective solid media.
4. Confirmation by cultural, biochemical and serological methods.

Precautions:
A rigorous control of enrichment incubation temperature is recommended because above 42ºC the growth rate of serotype O157 decreases dramatically.

Necessary supplements

Novobiocin Selective Supplement (Art. No. 06-139LYO1)
Vial Contents:
Necessary amount for 500 mL of complete medium.
Novobiocin, sodium salt..... 10,00 mg

Distilled water (Solvent)

Quality control

Incubation temperature: 41ºC ±0,5 **Incubation time:** 24 h

Inoculum: Practical range 100 ± 20 CFU. Min. 50 CFU (productivity)/ 10^4-10^6 CFU (selectivity) according to ISO 11133:2014/Amd 1:2018.

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC® 6538	Inhibited	Recovery in TSA
Enterococcus faecalis ATCC® 29212	Inhibited	Recovery in TSA
E. coli ser. O157:H7 ATCC® 700728 (non toxig.)	Good	Recovery in MacConkey Sorbitol Agar
E. coli ATCC® 8739	Inhibited	Recovery in MacConkey Sorbitol Agar
E. coli ATCC® 25922	Inhibited	Recovery in MacConkey Sorbitol Agar
Pseudomonas aeruginosa ATCC® 27853	Poor to fair	Recovery in MacConkey Sorbitol Agar

References

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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).