Trypticasein Soy Broth Modified with Novobiocin (mTSB) ISO

Cat. 1292

For the enrichment of enterohemorragic E.coli in foods

Practical information

Aplications	Categories
Selective enrichment	Escherichia coli O157

Industry: Food

Regulations: ISO 16654

Principles and uses

Trypticasein Soy Broth Modified with Novobiocin (mTSB) is recommended by ISO 16654 for the enrichment of E.coli O157:H7.

Casein peptone and soy peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Dipotassium phosphate acts as a buffer system. Glucose is the fermentable carbohydrate providing carbon and energy. Bile salts and Novobiocin are inhibitors of gram-positive organisms.

ISO 16654 recommends preparing the initial suspension and adding a test portion to the 41,5 ºC pre-warmed broth to obtain a ratio of test portion to mTSB + sample of 1/10 (mass to volume, or volume to volume). Incubate for 6 hours then a further 12 hours to 18 hours at 41,5 ºC.

Formula in g/L

Glucose	2,5	Bile salts N° 3	1,5
Dipotassium phosphate	4	Novobiocin	0,02
Pancreatic digest of casein	17	Sodium chloride	5
Soy peptone	3		

Preparation

Suspend 33 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes and sterilize in autoclave at 121 °C for 15 minutes.

Instructions for use

For the detection of Escherichia coli O157 according to ISO 16654:
- Prepare the initial suspension adding the test portion to Tripticasein Soy Broth Modified with Novobiacin (Cat. 1292) prewarmed to 41,5 ºC to obtain a ratio of 1/10.
- Separate and concentrate the microorganisms by immunogenic particles coated with antibodies to E. coli O157.
- Incubate for 6 hours then a further 12 hours to 18 hours at 41,5 ºC.
- Subculture the immunomagnetic particles with the bacteria adhered on MacConkey Agar with Sorbitol (Cat. 1099) and a second selective isolation agar of choice by the laboratory. The optimal incubation temperature for E. coli O157 is 37±1 °C for 18-24 hours.
- Confirm by indol production in Tryptophan Culture Broth (Cat. 1237) and agglutination with the serum anti E. coli O157.

Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Amber	7,4±0,2

Microbiological test

Incubation conditions: (41,5±1 ºC / 6;12-18 h).

Inoculation conditions: Productivity qualitative (<100 CFU) / Selectivity (10^4-10^6 CFU).

Microrganisms	Specification
Escherichia coli ATCC 25922	Good Growth
Escherichia coli 0157:H7 ATCC 43895	Good growth
Staphylococcus aureus ATCC 6538	Total inhibition

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

ISO 16654:2001 Microbiology of food and animal feed stuffs. Horizontal method for the detection of E. coli O157.
PHLS Standard Methods F17 & W16: Detection of E. coli O157.
FDA/BAM 8th Edition (Revision A) (1998) Chapter 4: 4.20-4.26.