

Tryptophan Culture Broth ISO

Cat. 1237

For the detection of *Escherichia coli* and other coliforms by indole production

Practical information

Applications	Categories
Detection	Coliforms
Detection	<i>Escherichia coli</i>

Industry: General cultivation

Regulations: ISO 16654



Principles and uses

Tryptophan Culture Broth ISO is used for the quick and standard test for the detection of *E. coli* and other coliforms by indole production. Indole formation depends exclusively on the development of bacteria producing enzymes called tryptophanases, which oxidize the L-tryptophan essential amino acid producing indole, skatole (methyl indole) and indole acetate. Several bacteria genera have species that are indole positive, particularly *Proteus*, *Escherichia*, *Edwardsiella*, *Flavobacterium*, *Aeromonas*, *Plesiomonas*, *Bacillus*, etc.

Tryptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. No fermentable carbohydrates in the medium allow for the good synthesis of tryptophanase and therefore indole production.

Formula in g/L

Pancreatic digest of casein	10	Sodium chloride	5
L-Tryptophan	1		

Preparation

Suspend 16 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. Distribute in test tubes, 3 ml in each. Close the tubes with cotton or with a plastic or metallic cap and sterilize in autoclave at 121°C for 15 minutes.

Instructions for use

For the detection and enumeration of *Escherichia coli* and coliform bacteria in water samples:

- Filter an appropriate volume of sample through the membrane.
- Place the membrane onto the surface of a TTC Chapman Agar (Cat. 1076) plate, avoiding the formation of air bubbles.
- Invert the plates and incubate at 36±2 °C for 21±3 hours.
- Count as lactose-positive bacteria the colonies that present a yellow development of the medium under the membrane.
- Subculture the characteristic colonies obtained, in non-selective agar and Tryptophan Culture Broth (Cat. 1237).
- Carry out the oxidase test and incubate the tubes of Tryptophan Culture Broth at 44±0,5 °C for 21±3 hours.
- Indole production is determined by adding a few drops of Kovac's Reagent (Cat. 5205) to the incubated Tryptophan Culture Broth tubes. A positive test is indicated by the development of red color in the reagent layer.
- The colonies that are oxidase negative will be considered as coliform bacteria and the colonies that are negative oxidase and positive indol will be considered as *E.coli*.

For the detection of *Escherichia coli* O157 according to ISO 16654:

- Prepare the initial suspension adding the test portion to Trypticasein Soy Broth Modified with Novobiacin (Cat. 1292) prewarmed to 41,5 °C 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 °C.
- 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 (25°C)
w/o rests	Fine powder	Beige	Amber, slightly opalescent	7,5 ± 0,1

Microbiological test

Incubation conditions: (44±5 °C / 21±3 h)

Microrganisms	Specification	Characteristic reaction
Klebsiella pneumoniae ATCC 13833	Good growth	Indole (-)
Escherichia coli ATCC 25922	Good growth	Indole (+)

Storage

Temp. Min.:2 °C
Temp. Max.:25 °C

Bibliography

ISO 9308-1. Water quality-Detection and enumeration of Escherichia coli and coliform bacteria. Part 1: Membrane filtration method
ISO 16654:2001 Microbiology of food and animal feeding stuffs. Horizontal method for the detection of Escherichia coli O157