Tryptone Soy Agar ISO

Cat. A2WWI0

For the detection and enumeration of E.coli in water.

Practical information

Aplications	Categories
Non selective enumeration	Coliforms
Non selective enumeration	Escherichia coli
Detection	Coliforms
Detection	Escherichia coli

Industry: Water

Regulations: ISO 9308

Principles and uses

Tryptone Soy Agar is used for the quick and standard test for the detection and count of E. coli and other coliforms by the membrane filter technique as directed by the ISO 9308-1.

It is also a general purpose solid medium, particularly useful for the isolation, sensitivity testing and determination of hemolysis with fastidious microorganisms as it does not contain any sugars. Another use of the Tryptone Soy Agar is for the oxidase test according to the traditional method as described in ISO 9308-1.

Casein and soy peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride maintains the osmotic balance and bacteriological agar is the solidifying agent.

Formula in g/L

Bacteriological agar	15	Sodium chloride	5
Soy peptone	5	Tryptic digest of casein	15

Preparation

Suspend 40 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 ºC for 15 minutes. Cool to 50 ºC, mix well and dispense into plates.

Instructions for use

For enumeration of E. coli and other coliform bacteria according to ISO 9308:

- Filter 100 ml of the sample through a membrane.
- Place the filter on a plate of Chromogenic E. coli Coliforms Agar (CCA) (Cat. 2080).
- Reverse the plate and incubate at 36±2 ºC for 21±3 hours.
- Count positive ß-D-galactosidase colonies (pink to red) as presumptive coliform bacteria other than E. coli.

To avoid possible false positives results, caused by positive oxidase bacteria such as Aeromonas spp, confirm the bacterial colonies through the oxidase test. Coliforms will be those that have an oxide-negative result.

- Count the ß-D-galactosidase and ß-D-glucuronidase positive (dark blue to violet) colonies as E. coli.
- If the colonies have not grown adequately to perform the oxidase test with pure colonies, it is necessary to subcultivate them in a non-selective medium such as Triptone Soy Agar (TSA) (Cat. 1138).
- Incubate plates in inverted position at 36±2 ºC for 21±3 hours.

The total coliform count is the sum of oxide-negative colonies, ß-D-galactosidase positive colonies (pink to red) and all colonies that are dark blue to violet.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Amber, slightly opalescent	7,2±0,1

Microbiological test

Incubation conditions: (36±2 ºC / 21±3 h).

Microorganisms	Specification
Klebsiella pneumoniae ATCC 13833	Good growth
Escherichia coli ATCC 25922	Good growth

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

ISO 9308-1 Water quality. Detection and enumeration of Escherichia coli and coliform bacteria. Part 1 Membrane filtration method
Regulation water quality- Detection and count of Escherichia coli and coliform bacteria. Anon. 1987 J. Food Microbiol., 5: 291 -296.