TBX Chromogenic Agar (Tryptone Bile X-Glucuronide) ISO

Cat. 1151

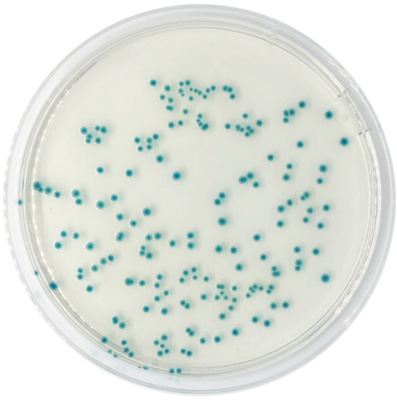
Selective medium for the detection and enumeration of Escherichia coli in foods.

Practical information

Aplications	Categories
Selective enumeration	Escherichia coli
Detection	Escherichia coli

Industry: Food

Regulations: ISO 11133 / ISO 16649



Principles and uses

TBX Chromogenic Agar (Tryptone Bile X-Glucuronide) is based on Tryptone Bile Salts Agar medium, used to detect and enumerate E. coli in foods, with the addition of a chromogenic agent, x-ß-D-Glucuronide, to detect the presence of the enzyme glucuronidase, which is highly specific for E. coli.

The released chromophore in TBX Agar is colored and target colonies are easily identified. E. coli absorbs the chromogenic agent x-ß-D-glucuronide, and the intracellular glucuronidase enzyme activity breaks the bond between the chromophore and the glucuronide. The released chromophore is colored and builds up within the cells, causing the E. coli colonies to be blue-green colored.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Bile Salts are inhibitors to other Gram-positive organisms and suppress coliform bacteria. Bacteriological agar is the solidifying agent.

ISO 16649 specifies a horizontal method for the enumeration of ß-glucuronidase-positive E. coli in products intended for human consumption or for the feeding of animals.

The negative b-ß-glucuronidase E. coli colonies are colorless, e.g. E. coli O157: H7. The high temperatures (44°C) inhibit the growth of E. coli O157: H7.

Formula in g/L

Enzymatic digest of casein	20	Bacteriological agar	15
Bile salts N° 3	1,5	5-bromo-4-cloro-3-indolil-ß-D-glucuronic acid	0,075

Preparation

Suspend 36,6 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. Sterilize in autoclave at 121 ºC for 15 minutes. Cool to 45-50 ºC, mix well and dispense into plates.

Instructions for use

Enumeration of ß-glucuronidase-positive Escherichia coli according to ISO 16649:
- Inoculate the TBX agar either by the plating method in depth, seeding on the surface or by the membrane filtration method.
- The membrane filtration method and the enumeration by the most probable number technique needs a previous resuscitation stage in Minerals Modified Glutamate Agar or Broth MMGA or MMGB (Cat. 1365).
- Incubate the plates of TBX agar for 21 hours at a temperature of 44 °C.
- Calculate the number of positive Escherichia coli ß-glucuronidase colonies from the number of typical blue colonies.

Quality control

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

Microbiological test

According to ISO 11133:
Incubation conditions: (44±1 ºC / 21±3 h).
Inoculation conditions: Productivity quantitative (100± Min. 50 CFU) / Productivity cualitative (10^3-10^4 CFU) / Selectivity (10^4-10^6 CFU) / Specificity (10^3-10^4 CFU).
Reference media: TBX.

Microorganisms	Specification	Characteristic reaction
Enterococcus faecalis ATCC 19433	Total inhibition (0)	
Escherichia coli ATCC 25922	Good growth (2) >50%	Blue colonies
Pseudomonas aeruginosa ATCC 27853		White to green-beige colonies
Enterococcus faecalis ATCC 29212	Total inhibition (0)	
Citrobacter freundii ATCC 43864		White to green-beige colonies
Escherichia coli ATCC 8739	Good growth (2) >50%	Blue colonies
Escherichia coli CECT 9153	Good growth (2) >50%	Blue colonies

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

International standard ISO 16649: Microbiology of food animal feeding stuffs. Horizontal method for the enumeration of presumptive ß-glucuronidase –positive.