

TECHNICAL DATA SHEET

Article no. : 8858.0500

TRYPTONE BILE GLUCURONIC AGAR (TBX AGAR)

ALSO KNOWN AS

TBX

SPECIFICATION

Selective and differential medium for the detection and enumeration of β -glucuronidase (+) *E. coli* according to ISO standards.

FORMULA * IN G/L

Tryptone.....	20.000
Bile salts No. 3.....	1.500
5-Bromo-4-chloro-3- indoxyl- β -D-glucuronide.....	0.075
Agar.....	15.000

Final pH 7.2 \pm 0.2 at 25 °C

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

DIRECTIONS

Add 36.5 g of the powder in 1 L of distilled water and heat to boiling with continuous stirring until total dissolution. Dispense into suitable containers and sterilize in the autoclave at 121 °C for 15 minutes.

DESCRIPTION

Escherichia coli is the only coliform that possesses β -D-glucuronidase and can be easily differentiated from other coliforms that do not show this enzymatic activity. There are some strains of *E. coli* (less than 3-4% of the total population) that are β -D-glucuronidase negative.

E. coli absorbs the chromogenic substrate (X- β -D-glucuronide) and the bacterial enzyme β -D-glucuronidase splits the bond between the chromophoric X-fraction and the β -D-glucuronide.

The free X-fraction dyes the *E. coli* cells and produces a blue-green colony.

The high content in bile salts of the medium inhibits the growth of accompanying Gram positive bacteria and the high incubation temperature (44 \pm 1 °C) inhibits Gram negative bacteria other than *E. coli*.



TECHNIQUE

1. Direct inoculation (Pour plate technique)

Transfer 1 ml of test sample to a sterile Petri dish aseptically, and repeat the procedure with further dilutions. Inoculate two plates per dilution. Pour 15 ml of melted and cooled (44-47 °C) TBX Agar into each Petri dish. Mix carefully and allow the mixture to solidify. The time between the distribution of the inoculum and pouring the medium should not exceed 15 minutes.

Invert the inoculated plates and incubate them at 44±1 °C for 20-24 hours. If the presence of stressed cells is suspected incubate for an initial period of 4 h ±0.25 at 37±1 °C and then raise the incubation temperature to 44 °C. The total incubation time should not exceed 24 hours and the incubation temperature should not exceed 45 °C.

2. Membrane incubation (Resuscitation technique)

No special membranes are recommended. Any sterile and non-inhibitive membrane made of cellulose acetate or mixed esters of cellulose, with 0.45 µm to 1.2 µm pore size and 85 mm diameter can be used.

2.1. Resuscitation

Aseptically place a membrane on the dried surface of each of two plates of Mineral-Modified-Glutamate Agar (MMGA) with care to avoid trapping air bubbles. Add 1 ml of the test sample to the centre of each membrane and spread the inoculum evenly over the whole membrane surface. Repeat the procedure for each dilution of the sample. Leave the inoculated plates at room temperature for 15 minutes until the inoculum has soaked into the agar. Incubate the plates at 37±1 °C for 4 ± 0.25 hours.

2.2. Transfer to the selective medium

After the resuscitation period, transfer the membranes from the resuscitation medium to the plates of TBX Agar using sterile forceps, taking care to avoid trapping air bubbles beneath the membrane. Do not touch nor disturb the membrane surface. Incubate the plates for 20-24 hours at 44 °C (and not more than 45 °C).

3. Results

The β-D-glucuronidase-positive *Escherichia coli* produces blue colonies (Blue-green). Some strains (3-4 % of the total population) of *E. coli* lack the glucuronidase enzyme and produce colourless colonies. Some stressed cells of *E. coli* are unable to grow at 44 °C and do not produce colonies.

QUALITY CONTROL

Incubation Temperature: 44 ± 1 °C

Incubation Time: 20-24 h

Inoculum: Practical range 100±20 CFU. min. 50 CFU (productivity)/ 10⁴-10⁶ CFU (selectivity)/ 10³-10⁴ CFU (specificity), according to ISO 11133:2014/Amd 1:2018. MF method.

Microorganism	Growth	Remarks
<i>Enterococcus faecalis</i> ATCC® 19433	Inhibited	Selectivity
<i>Escherichia coli</i> ATCC® 25922	Productivity > 0.50	Blue colonies
<i>Escherichia coli</i> ATCC® 8739	Productivity > 0.50	Blue colonies
<i>E. coli</i> NCTC® 13216	Productivity > 0.50	Blue colonies
<i>C.freundii</i> ATCC® 43864	Good	Colorless colonies

REFERENCES

- DELISLE, G.L. & A. LEY (1989) Rapid detection of *E. coli* in urine samples by a new chromogenic β-glucuronidase assay. *J. Clin. Microbiol.* 27:778-779
- ISO Standard 16649-1:2018. Microbiology of foods chain- Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* - Part 1: Colony count technique at 44°C using membranes and 5-bromo-4-chloro-3-indolyl β-D-glucoride.
- ISO 11133:2014. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- OGDEN, I.D. & A.J. WATT (1991) An evaluation of fluorogenic and chromogenic assays for the direct enumeration of *E. coli*. *Letters in Appl. Microbiol.* 13:212-215.
- SCHWEIZERISCHES LEBENSMITTELBUCH (2005) Kap.56 Mikrobiologie, Bundesamt für Gesundheit. Direktionsbereich Verbraucherschutz. Bern.

STORAGE

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

