**Product :**

# Microinstant ® TRYPTONE BILE GLUCURONIC A. (TBX AGAR)

## Also known as

TBX

## Specification

Selective and differential medium for the detection and enumeration of ß-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-ß-D-glucuronide | 0.075 |
| Agar | 15.000 |

Final pH 7.2 ±0.2 at 25 ºC

* 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 ºC for 15 minutes.

## Description

*Escherichia coli* is the only coliform that possesses ß-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 ß-D-glucuronidase negative.

*E. coli* absorbs the chromogenic substrate (X-ß-D-glucuronide) and the bacterial enzyme ß-D-glucuronidase splits the bond between the chromophoric X-fraction and the ß-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±1 ºC) 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 ºC) 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 à 44±1 ºC for 20-24 hours. If the presence of stressed cells is suspected incubate for an initial period of 4 h ±0.25 à 37±1 ºC and then raise the incubation temperature to 44 ºC. The total incubation time should not exceed 24 hours and the incubation temperature should not exceed 45 ºC.

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 à room temperature for 15 minutes until the inoculum has soaked into the agar. Incubate the plates à 37±1 ºC 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 à 44 ºC (and not more than 45 ºC).

3. Results

The ß-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 à 44 ºC and do not produces colonies.

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## Quality control

**Incubation temperature:** 44 ± 1 ºC **Incubation time:** 20-24 h

**Inoculum:** Practical range 100±20 CFU. min. 50 CFU (productivity)/ $10^4$ -10□ CFU (selectivity)/ ≥ $10^3$ CFU (specificity), according to ISO 11133:2014/Amd 1:2018. MF method.

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

## References


· DELISLE, G.L. & A. LEY (1989) Rapid detection of E. coli in urine samples by a new chromogenic ß-glucuronidase assay. J. Clin. Microbiol. 27:778-779

· ISO Standard 16649-1:2018. Microbiology of foods chain- Horizontal method for the enumeration of ß-glucuronidase-positive *Escherichia coli* - Part 1: Colony count technique at 44ºC using membranes and 5-bromo-4-chloro-3-indolyl ß-D-glucoride.

. ISO 11133:2014/ Adm 1:2018. 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 ºC to 30 ºC).