

TECHNICAL DATA SHEET

Article No. 9880

TBX Agar, ready-to-use culture medium

SYNONYMS

Tryptone Bile x-Glucuronide agar, , Tryptone Bile Glucuronide Agar

SPECIFICATION

Prepared medium. Selective and differential solid medium for the detection and enumeration of β -glucuronidase positive *Escherichia coli* according to ISO 16649.

Colour: Straw-coloured yellow
pH: 7.2 \pm 0.2 at 25 °C

COMPOSITION IN G/L

Tryptone	20.000
Bile Salts	1.500
Agar	15.000
5-Bromo-4-chloro-3-indoxyl- β -D-glucuronide	0.075

PACKAGING DETAILS

9880-10x100ML

10 prepared bottles

Volume: 125 \pm 3 ml

Packaging unit: 1 box with 10 125-ml-bottles. Plastic screw inner cap. For use with syringe needles with a diameter \leq 0.8 mm.

9880-10x200ML

10 prepared bottles

Volume: 200 \pm 5 ml

Packaging unit: 1 box with 10 250-ml-bottles. Plastic screw inner cap. For use with syringe needles with a diameter \leq 0.8 mm.



GUIDELINES

Description:

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

Escherichia 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 *Escherichia 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°C) inhibits Gram negative bacteria other than *Escherichia coli*.

Technique:

Melt the Agar (100°C), pour into plates and proceed according to internal specifications.

1. Direct inoculation (Pour plate technique)

Aseptically transfer 1 ml of test sample to a sterile petri dish, 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 $4 \text{ h} \pm 0.25$ 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 center 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 *Escherichia coli* lack the glucuronidase enzyme and produce colourless colonies. Some stressed cells of *Escherichia coli* are unable to grow at 44°C and produces no colonies.

Note: The solid mediums can be melted in different ways: autoclave, bath and, if the customer considers appropriate, also the microwave. Whenever the microwave option is chosen, it is necessary to take certain safety measures to avoid breaking of the containers, such as loosening the screw cap and putting the bottle or tube in a water bath in the microwave. The fusion temperature and time will depend on the shape of the container, the volume of medium and the heat source. Avoid overheating as both the heating periods.



MICROBIOLOGICAL CONTROL

Melting - pour plates - inoculation
Practical range 100 ± 20 CFU. min. 50 CFU (productivity) / 10⁴-10⁶ CFU (selectivity)
Microbiological control according to ISO 11133:2014/A1:2018.
Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020
Aerobiosis. Incubate at 44 °C ± 1 °C for 20 - 24 h

Microorganism	Growth
<i>Escherichia coli</i> ATCC® 25922, WDCM 00013	Good (≥ 50%) blue colonies
<i>Escherichia coli</i> ATCC® 8739, WDCM 00012	Good (≥ 50%) blue colonies
<i>Escherichia coli</i> NCTC® 13216, WDCM 00202	Good (≥ 50%) blue colonies
<i>Enterococcus faecalis</i> ATCC® 19433, WDCM 00009	Inhibited
<i>Citrobacter freundii</i> ATCC® 43864 (37°C ±1)	Good - colourless colonies

Sterility control:

Incubation 48 hours at 30-35 °C and 48 hours at 20-25 °C: NO GROWTH.
Check at 7 days after incubation in same conditions.

BIBLIOGRAPHY

- 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-glucuronide. 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

8-25 °C

SHELF LIFE

12 months unopened from date of manufacture

updated: 30.08.2022

