ALLIANCE BIO EXPERTISE

Reference: FLWR3093 **A.B.E. - Technical Data Sheet**

Product: **TBX Agar - 200 ml**

Specification

Selective and differential solid medium for the detection and enumeration of ß-glucuronidasepositive *Escherichia coli* according to ISO standards.

Presentation

	Packaging Details	Shelf Life	Storage
10 Prepared bottles Bottle 250 ml with: 200 ± 5 ml	1 box with 10 bottles 250 ml. Injectable cap: Plastic screw inner cap. The use of syringes needles with a diameter greater than 0.8 mm is not recommended	12 months	2-25 ºC

Composition

Composition (g/l):

Tryptone	20.000
Bile salts no 3	1.500
Agar	15.000
5-Bromo-4-chloro-3-indoxyl-ß-D-glucuronide	0.075

Description /Technique

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 (44ºC) inhibits Gram negative bacteria other than *E. coli*.

Directions for Use:

Melt the tube (100ºC), pour into the plate and proceed according to internal specifications.

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 (44-47ºC) 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±1ºC for 20-24 hours. If the presence of stressed cells is suspected incubate for an initial period of 4h ±0,25 at 37±1ºC and then raise the incubation temperature to 44ºC. The total incubation time should not exceed 24 hours and the incubation temperature should not exceed 45ºC.

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 ±1ºC 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 44ºC (and not more than 45ºC).

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 at 44ºC 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.

Quality control

Physical/Chemical control

Color : Straw-coloured yellow pH: 7.2 ± 0.2 at 25ºC

Microbiological control

Melting - pour plates - inoculation Practical range 100 ± 20 CFU. min. 50 CFU (productivity) / 10^4-10^6 CFU (selectivity)

Microbiological control according to ISO 11133:2014/A1:2018.

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Aerobiosi. Incubate at 44 ºC ± 1 ºC for 20 - 24 h

Microorganism	Growth
Escherichia coli ATCC® 25922, WDCM 00013	Good (≥ 50%) Blue colonie
Escherichia coli ATCC® 8739, WDCM 00012	Good (≥ 50%) Blue colonie
Escherichia coli NCTC® 13216, WDCM 00202	Good (≥ 50%) Blue colonie
Enterococcus faecalis ATCC® 19433, WDCM 00009	Inhibited
Citrobacter freundii ATCC® 43864, WDCM 00006 (37/44ºC)	Good - Colourless colonies

Sterility control

Incubation 48 h at 30-35 ºC and 48 h at 20-25 ºC: 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 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.
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· 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.

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