

Tryptone bile x-glucuronide agar - TBX (ISO)**Code 84637.0500****Also known as**

TBX agar

Intended useFor the enumeration of β -glucuronidase positive *E. coli* in food and animal feeding stuffs (ISO 16649).**Formula * - Composition in g/L**

Enzymatic digest of casein.....	20.000
Bile Salts No. 3.....	1.500
5-Bromo-4-chloro-3-indolyl β - D-glucuronic acid (BCIG).....	0.075
Agar.....	14.000

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

Final pH 7.2 \pm 0.2 at 25 °C.**Instructions for preparation**

Dissolve 36.6 g in 1 litre of purified water by bringing to the boil with frequent shaking. Sterilise in the autoclave at 121 °C for 15 minutes.

Principle of the method and general information

Tryptone bile x-glucuronide -TBX agar is a selective and chromogenic medium for the enumeration of β -glucuronidase positive *Escherichia coli* in food and animal feeding stuffs. It is a modification of Tryptone bile agar and is prepared according to the formulation recommended by ISO 16649-1 and ISO 16649-1. TBX Agar must be used with an incubation temperature of 44°C both with poured plated method (ISO 16649-2) and with membrane filtration technique (ISO 16649-1)

The medium contains enzymatic digest of casein that supplies nitrogen and carbon for bacterial growth, bile salts for the inhibition of Gram-positive bacteria and the chromogenic compound 5-bromo-4-chloro-3 indolyl β -D-glucuronide (BCIG) for the detection of β -glucuronidase. The bacterial enzyme splits the bond between 5-bromo-4-chloro-3-indolyle and β -D-glucuronide: the released chromophore is coloured and accumulates within the cells and the colonies.

Among the *Enterobacteriaceae*, *E. coli* and some strains of *Salmonella* and *Shigella*, are β -glucuronidase positive, so cultivates on the plates with blue colonies. β -glucuronidase negative bacteria grow with colourless colonies.

Instruction for use

For laboratory use only.

Prepare the test portion, initial suspension, and further dilution of the sample according to the specific International Standard appropriate to the product concerned.

- Transfer 1 ml of the test sample in duplicate, into two sterile Petri dishes if liquid, or 1ml of the initial suspension (10^{-1}), in the case of other products. Repeat the procedure with further decimal dilutions if necessary.
- Within 15 minutes, pour into each Petri dish about 15 ml of TBX agar pre-cooled to 44-47 °C.
- Mix well the inoculum with the medium. Invert the inoculated dishes and incubate at 44 °C for 18-24 hours. In cases where stressed colonies are suspected incubate for 4 hours at 37°C before incubation at 44 °C. Do not incubate over 45°C.
- Count as β -glucuronidase positive *E.coli* the blue colonies in the plates with less than 150 typical colonies and less than 300 total colonies (typical and atypical)
- Report the results as CFU/g considering the "dilution factor" and according to the recommendations of ISO norms.

Consult the quoted ISO Standards for the details of the procedures for enumeration of *E.coli* by poured plate method or by membrane filtration method.

Limitations

- It is recommended that biochemical and/or serological tests be performed on pure culture for complete identification.
- Some *Salmonella* and *Shigella* strains that are β -glucuronidase positive may be recognized on TBX agar as β -glucuronidase positive *E.coli*.

Quality Control:

Physical characteristics:

Appearance of powder

Beige, fine, homogeneous, hygroscopic powder

Appearance of prepared medium

Beige, limpid

pH (25°C)

7.2 \pm 0.2

Microbiological characteristics:

Test Strains	Incubation T° / t / At.	Inoculation method	Growth characteristics	Productivity ratio
<i>E. coli</i> ATCC 25922	QT	44 °C / 24 h / AE	Blue colonies	PR \geq 0.5
<i>E. coli</i> ATCC 8739	QT	44 °C / 24 h / AE	Blue colonies	PR \geq 0.5
<i>C. freundii</i> ATCC 43864	EC	44 °C / 24 h / AE	Colourless colonies	
<i>K. pneumoniae</i> ATCC 27736	EC	44 °C / 24 h / AE	Mucoid colourless colonies	
<i>E. faecalis</i> ATCC 29212	MM	44 °C / 24 h / AE	Totally inhibited	

Notes

PR (Productivity Ratio): CFU obtained on the culture medium under test / CFU obtained on Tryptic soy agar

Incubation atmosphere AE: aerobic incubation

Inoculation method QT : quantitative surface plating method; EC: semi-quantitative, ecometric technique; MM: modified Miles-Misra surface drop method

Microbiological characteristics tested in accordance to ISO/TS 11133-2

ATCC is a registered trade mark of American Type Culture Collection

References

- ISO 16649-2 (2001). Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive *E.coli*- part 2: colony count technique at 44°C using 5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid.
- ISO 16649-1 (2001). Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive *E.coli*- part 2: colony count technique at 44°C using membranes and 5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid.

Storage conditions

For laboratory use only. Keep tightly closed, away from bright light at +2 °C to 8 °C and <60% RH.

Ordering information

84637.0500 Tryptone bile x-glucuronide agar - TBX (ISO) Bottle of 500 g