CHROMOGENIC TRYPTONE BILE X-GLUCURONIDE (TBX) AGAR

AGTB-00I-500

- **Principle**

TBX Chromogenic Agar (Tryptone Bile X-Glucuronide) is based on Tryptone Bile Salts Agar medium, used to detect and enumerate *E. coli* in foods, with the addition of a chromogenic agent, x-ß-D-Glucuronide, to detect the presence of the enzyme glucuronidase, which is highly specific for *E. coli*.

The released chromophore in TBX Agar is coloured and target colonies are easily identified. *E. coli* absorbs the chromogenic agent x-ß-D-glucuronide, and the intracellular glucuronidase enzyme activity breaks the bond between the chromophore and the glucuronide. The released chromophore is coloured and builds up within the cells, causing the *E. coli* colonies to be blue-green coloured.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Bile Salts are inhibitors of other Gram-positive organisms and suppress coliform bacteria. Bacteriological agar is the solidifying agent.

ISO 16649 specifies a horizontal method for the enumeration of ß-glucuronidase-positive *E. coli* in products intended for human consumption or for the feeding of animals.

The negative b-ß-glucuronidase *E. coli* colonies are colourless, e.g. *E. coli* O157: H7. The high temperatures (44°C) inhibit the growth of *E. coli* O157: H7.

- **Regulatory compliance**

This product is manufactured under a quality management system in accordance with ISO 9001 and ISO 13485, and its formulation and quality control comply with applicable international standards, such as ISO 11133, where relevant.

For this specific medium, compliance is also established with the relevant requirements of ISO 16649.

- **Composition**

Ingredients	**g/L**
Enzymatic digest of casein	20.00
Bile salts N° 3	1.50
Bacteriological agar	15.00
5-bromo-4-cloro-3-indolil-ß-D-glucuronic acid	0.075

- **Preparation**

Suspend 36.6 grams of the medium in one litre of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 °C for 15 minutes. Cool to 45-50 °C, mix well and dispense into plates.

- **Applications and use**

Enumeration of ß-glucuronidase-positive *Escherichia coli* according to ISO 16649:

- Inoculate the TBX agar either by the plating method in depth, seeding on the surface or by the membrane filtration method.

- The membrane filtration method and the enumeration by the most probable number technique needs a previous resuscitation stage in minerals.

- **Quality control**

Solubility	w/o rests
Appearance	Fine powder
Colour of the dehydrated medium	Beige
Colour of the prepared medium	Amber
Final pH (25 °C)	7.2 ± 0.2

- **Microbiological test**

According to ISO 11133:

Incubation conditions: 44±1 °C / 21±3 h.

Inoculation conditions: Productivity quantitative (100± Min. 50 CFU) / Productivity qualitative (10^3-10^4 CFU) / Selectivity (10^4-10^6 CFU) / Specificity (10^3-10^4 CFU). Inoculum 50-100 CFU.

Reference media: TBX.

Microorganisms	ATCC	Specification	Characteristic reaction
Enterococcus faecalis	19433	Total inhibition (0)	-
Escherichia coli	25922	Good growth (2) >50%	Blue colonies
Pseudomonas aeruginosa	27853	-	White to green-beige colonies
Enterococcus faecalis	2912	Total inhibition (0)	-
Citrobacter freundii	43864	-	White to green-beige colonies
Escherichia coli	8739	Good growth (2) >50%	Blue colonies
Escherichia coli	9153 (CECT)	Good growth (2) >50%	Blue colonies

- **Storage**

The product is highly hygroscopic; keep the container always closed and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Temp. Min.:2 °C Temp. Max.:25 °C.

Note: Sterilize media immediately after reconstitution.

- **Bibliography**

International standard ISO 16649: Microbiology of food animal feeding stuffs. Horizontal method for the enumeration of presumptive ß-glucuronidase –positive.

- **Product use limitation**

This product is developed, designed and supplied exclusively for research use only. It is not intended for diagnostic applications or drug development, and it is not suitable for administration to humans or animals.