

T.B.X. (Tryptone Bile X-Glucuronide) CHROMOGENIC AGAR

(ISO 16449-2)

 Chromogenic medium for detection and enumeration of *E. coli*, according to ISO 16649

DESCRIPTION

 Selective medium for the detection and enumeration of *Escherichia coli* in foods.

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-β-D-Glucuronide, to detect the presence of the enzyme glucuronidase, which is highly specific for *E. coli*. The released chromophore in TBX Agar is colored and target colonies are easily identified.

E. coli absorbs the chromogenic agent x-β-D-glucuronide, and the intracellular glucuronidase enzyme activity breaks the bond between the chromophore and the glucuronide. The released chromophore is colored and builds up within the cells, causing the *E. coli* colonies to be blue-green colored. Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Bile Salts are inhibitors to other Gram-positive organisms and suppress coliform bacteria. Bacteriological agar is the solidifying agent.

ISO 16649 specifies a horizontal method for the enumeration of β-glucuronidase-positive *E. coli* in products intended for human consumption or for the feeding of animals. The negative β-glucuronidase *E. coli* colonies are colorless, e.g. *E. coli* O157: H7. The high temperatures (44°C) inhibit the growth of *E. coli* O157: H7.

COMPOSITION

	g/L
Enzymatic Digest of Casein	20.0
Bile Salts No. 3	1.5
X-Glucuronide	0.075
Agar	15.0

Final pH 7,0 ± 0,2 at 25°C

WARNING AND PRECAUTIONS

For in vitro diagnostic use.

Observe the precautions normally taken when handling laboratory reagents.

Dehydrated medium: HIGHLY HYGROSCOPIC. During the handling, wear dust protection mask. Avoid the eye contact. Do not use beyond the expiration date or if the product shows signs of deterioration, an altered color or has compacted.

Prepared Medium: The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous.

Safety Data Sheet is available on request for professional users.

All waste must be disposed of according to local directives.

STORAGE AND STABILITY

Dehydrated medium:	2-8°C
Prepared medium:	2-8°C

The product is stable until the expiration date indicated on the label under the recommended storage conditions.

PREPARATION

Dehydrated medium: Suspend 36.6 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Sterilize in autoclave at 121°C for 15 minutes.

Prepared medium (bottles): Melt the content of the bottle in a water bath at 100°C until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into Petri dishes.

PROCEDURE

ISO 16649-1 recommends the following procedure:

- Place a filter membrane onto two plates of Mineral-Modified Glutamate Agar (MMGA) and spread 1 ml of the test sample over the whole membrane surface. Repeat the procedure with the further decimal dilutions, if necessary. Leave inoculated plates at room temperature for 15 min and incubate at 37°C for 4 ± 1 hours.
- After the resuscitation period transfer the membranes to TBX Agar plates and incubate at 44°C for 18-24 h. Alternatively, direct inoculation methods, either pour plate method or surface plate technique, can be used. For the recovery of

sub-lethally injured *E. coli*, incubate plates at 37°C or 30°C for 4 hours. Continue incubation at 44°C for additional 18-20 hours.

RESULTS

MICROORGANISM	TYPICAL COLONY COLOR
β-glucuronidase-positive <i>Escherichia coli</i>	Blue to blue-green
β-glucuronidase-negative bacteria (if not inhibited)	White to green-beige

QUALITY CONTROL

Dehydrated medium: free-flowing, homogeneous, light beige.

Prepared medium: slightly opalescent, colorless to light beige.

Typical response after incubation at 44±1°C for 18-24 hours, in aerobiosis

MICROORGANISM	GROWTH
<i>Escherichia coli</i> WDCM 00013	Good -Blue to blue-green colonies
<i>Escherichia coli</i> WDCM 00202	Good -Blue to blue-green colonies
<i>Enterococcus faecalis</i> WDCM 00009	Inhibited
<i>Citrobacter freundii</i> WDCM 00006	Good -White to green-beige colonies
<i>Pseudomonas aeruginosa</i> WDCM 00025	Good -White to green-beige colonies

REFERENCES

- International Standard ISO 16649-2:2001 Microbiology of food animal feeding stuffs. Horizontal method for the enumeration of presumptive *Escherichia coli*.
- UNI EN ISO 16649-2:2010 – Microbiology of food and animal feeding stuffs – Horizontal Method for the enumeration of beta-glucuronidase-positive *Escherichia coli* Colony-count Technique at 44°C using 5-bromo-4-chloro-3-indolyl-beta-D-glucuronide.

PRESENTATION

	Packaging	REF.
Dehydrated medium:		
T.B.X. CHROMOGENIC AGAR	500 g (13.6 L)	10310
Prepared medium:		
T.B.X. CHROMOGENIC AGAR	6 x 100 mL bottles	63318
	6 x 200 mL bottles	63218
	20 pcs (60 mm ready-to-use plates)	2790106/20
	20 pcs (90 mm ready-to-use plates)	2924650/20
	20 pcs RODAC Plates	31008

SYMBOLS

	Read the instructions		Biological hazard
	CE Mark (product complies with the requirements of Regulation (EU) 746/2017)		
	Temperature limitation		Use by
	For in vitro diagnostic use		Manufacturer