Technical Data Sheet

Chromocult TBX (Tryptone Bile X-glucuronide) Agar acc. ISO 16649

Ordering number: 1.16122.0500

For the enumeration, detection and identification of β-glucuronidase positive *Escherichia coli* from food and feeding stuff as well as from environmental samples in the area of food production and food handling.

This culture medium complies with the specifications given by ISO 16649 part 1 and part 2 and with those given by EN ISO 16649-3.

Mode of ction

The presence of the enzyme β-D-glucuronidase differentiates most *Escherichia coli* from other coliforms. *Escherichia coli* absorbs the chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-β-D-glucuronide). The enzyme β-glucuronidase splits the bond between the chromophore 5-bromo-4-chloro-3-indolyle- and the β-D-glucuronide. *Escherichia coli* colonies are then colored blue to blue-green.

Growth of accompanying Gram-positive flora is largely inhibited by the use of bile salts and the high incubation temperature of 44 °C.

Some *Escherichia coli* (3-4 %) are β-D-glucuronidase-negative and appear as colorless colonies, e.g. most *Escherichia coli* O157 strains or they cannot grow at the elevated temperature of 44 °C, e.g. *Escherichia coli* 0157:H7. For the detection of *Escherichia coli* O157 specific culture media have to be used.

Typical Composition

Specified by ISO 16649		**Chromocult TBX (Tryptone Bile X-glucuronide) agar acc. ISO 16649**	
Enzymatic Digest of Casein	20 g/l	Enzymatic Digest of Casein	20 g/l
Bile Salts No. 3	1.5 g/l	Bile Salts No. 3	1.5 g/l
5-Bromo-4-chloro-3-indolyl-β-D-glucuronic acid (BCIG) Cyclohexylammonium Salt*	0.075 g/l	5-Bromo-4-chloro-3-indolyl-β-D-glucuronic acid (BCIG) Cyclohexylammonium Salt	0.075 g/l
Agar	9-18 g/l	Agar-Agar**	10 g/l
Water	1000 ml/l	Water	n/a
pH at 25 °C	7.2 0.2	pH at 25 °C	7.2 0.2

* ISO 16649 specifies: 5-Bromo-4-chloro-3-indolyl-ß-D-glucuronic acid (BCIG) 144 µmol - for example 0.075 g of

cyclohexylammonium salt.
** Agar-Agar is equivalent to other different terms of agar.

Preparation

Dissolve 31.6 g in 1 l of purified water. Heat in boiling water, and agitate frequently until completely dissolved. Autoclave 15 minutes at 121 °C. After the medium has cooled to 45-50 °C pour the medium into plates or use it for the poured plate method.

The prepared medium is clear and yellowish to yellowish-brown.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Incubate the inoculated plates under aerobic conditions. e.g. acc. to ISO 16649 at 43-45 ° C for 20-24 h.

The presence of blue or blue green colonies indicates the presence of β-glucuronidase positive *E. coli.*

Colony-count technique using membranes and TBX agar acc. to ISO 16649-1:

The method is suitable for the enumeration of cells of *E. coli* that might have been

subjected to stress.

Using sterile forceps, aseptically place a sterile cellulose membrane with a pore size of 0.45 µm and a diameter of 85 mm onto the dried surface of each of two plates Mineral-modified glutamate agar (MMGA) (article number 1.09045.0500), taking care to avoid trapping air bubbles beneath the membranes. Gently flatten the membranes with a sterile spreader, if necessary.

Using a sterile pipette, add 1 ml of the test sample or the initial suspension to the centre of each membrane. Using a sterile spreader, spread the inoculum evenly over the whole membrane surface, avoiding any spillage from the membrane.

Repeat the procedure with the further decimal dilutions. If necessary, using another sterile pipette and another sterile spreader for each dilution.

Leave the inoculated plates in a horizontal position at room temperature for approximately 15 min until the inoculum has soaked through the membrane into the agar.

Incubate the plates at36-38 °C for 3-5 h, with the membrane/agar surface uppermost.

After resuscitation, transfer membranes using sterile forceps from MMGA (resuscitation medium) to plates of TBX agar.

Incubate the TBX plates at 43-45 °C for 20-24 h, with the membrane/agar surface uppermost. Do not stack dishes more than three high.

Examinate the TBX agar for the presence of blue or blue green colonies, indicating the presence of β-glucuronidase positive *E. coli* and count the typical CFU (colony-forming units).

Colony-count technique using poured plate method with TBX agar acc. to ISO 16649-2:

If the presence of stressed cells is suspected, incubate for an initial period of 4 h at 37 °C, and then raise the incubation temperature to 43-45 °C for 20-24 h.

Using a sterile pipette, transfer to a sterile Petri dish 1 ml of the test sample or 1 ml of the initial dilution. Inoculate two plates per dilution. Repeat the procedure with the further decimal dilutions, if necessary, using a new sterile pipette for each dilution.

Pour into each Petri dish approximately 15 ml of the TBX agar, previously cooled to 44-47 °C in the water batch.

Carefully mix the inoculum with the medium and allow the mixture to solidify, with the Petri dish standing on a cool horizontal surface.

The time which elapses between the distribution of the inoculum in a dish and pouring of the medium shall not exceed 15 min.

Invert the inoculated dishes, so that the bottom is uppermost and incubate them 43-45 ° C for 20-24 h. The total incubation shall not be longer than 24 h.

Examine the TBX agar for the presence of blue or blue green colonies, indicating the presence of of β-glucuronidase positive *E. coli* and count the typical CFU (colony-forming units).

Detection and most probable number (MPN) technique acc. to EN ISO 16649-3:

This method is suitable for the enumeration of cells of *E. coli* that might have been subjected to stress arising from dehydration, freezing, and exposure to a saline (such as marine) environment or damage by disinfectants such as chlorine-containing products.

For the detection method, Minerals modified glutamate medium (selective enrichment medium) is inoculated with a specified quantity of test sample if the initial product is liquid or with a specified quantity of the initial suspension in the case of other products.

The tube is incubated at 36-38 °C for 22-26 h. The tube is examined for acid production, indicating lactose fermentation.

If the tube has given rise to acid production, it is subcultured onto TBX agar.

Incubation of the TBX agar at 43-45 °C for 20-24 h.

Examination of the TBX agar for the presence of blue or blue green colonies, indicating the presence of β-glucuronidase positive *E. coli.*

The result is expressed as *E. coli* detected or not detected in x g or x ml of product.

For the enumeration method by MPN, inoculation of three or five tubes of double strength Minerals modified glutamate medium (selective enrichment medium) with an equal volume of the test sample if the initial product is liquid, or with an equal volume of the initial suspension in the case of other products. For live bivalve molluscs or other products requiring greater precision, it is necessary to inoculate a series of five tubes.

Inoculation of three or five tubes of single strength liquid enrichment medium with a specified quantity of test sample if the initial product is liquid, or with a specified quantity of the initial suspension in the case of other products.

Then, under the same conditions, inoculation of the medium with decimal dilutions of the test sample or of the initial suspension.

Incubation of the tubes of double strength and single strength medium at 36-38 °C for 22-26 h.

Examination of the tubes for acid production, indicating lactose fermentation.

For each tube of medium showing acid production, subculture to TBX agar.

Incubation of the TBX agar at 43-45 °C for 20-24 h.

Examination of the TBX agar for the presence of blue or blue green colonies, indicating the presence of β-glucuronidase positive E. coli.

Determination of the most probable number of β-glucuronidase positive *E. coli* from the number of tubes of medium that produced blue to blue green colonies after subculture to TBX agar, according to EN ISO 7218.

Storage

Store at +15 °C to +25 °C, dry and tightly closed. Do not use clumped or discolored medium. Protect from UV light (including sun light). For *in vitro* use only.

According to EN ISO 16649-3, self-prepared plates may be stored at +2 °C to + 8 °C in the dark and protected from desiccation for up to four weeks unless results of the laboratory shelf life validation indicate a longer shelf-life.

Quality Control

Function	Control strains	Incubation	Method of control	Expected results
Productivity	*Escherichia coli* ATCC® 8739	18-24 h at 43-45 °C	Quantitative	Recovery > 50 %, turquoise to blue colonies
	Escherichia coli ATCC® 25922			
	Escherichia coli NCTC 13216			
Selectivity	*Enterococcus faecalis* ATCC® 19433	18-24 h at 43-45 °C	Qualitative	Total inhibition
	Enterococcus faecalis ATCC® 29212			
Specificity	*Citrobacter freundii* ATCC® 43864	18-24 h at 43-45 °C	Qualitative	White to green-beige colonies
	Pseudomonas aeruginosa ATCC® 27853			

Please refer to the actual batch related Certificate of Analysis.

The performance test is in accordance with the current version of EN ISO 11133. A

recovery rate of 50 % is equivalent to a productivity value of 0.5.

Escherichia coli ATCC® 25922

Literature

Blazko N. (1988): Evaluation of the β-glucuronidase substrate 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide in a 24 hour direct plating method for *Escherichia coli.* J. Food Prot. **51:** 402.

ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* - Part 1: Colony-count technique at 44 °C using membranes and 5-bromo-4-chloro-3-indolyl beta-D-glucuronide. ISO 16649-1:2001.

ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* - Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide. ISO 16649-2:2001.

ISO International Standardisation Organisation. Microbiology of the food chain - Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* - Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide. EN ISO 16649-3:2015.

ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations. EN ISO 7218:2003.

ISO International Standardisation Organisation. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media. EN ISO 11133:2014.

Ley A.N., Bowers R.J. and Wolfe S. (1988): Indoxyl- β -D-glucuronide, a novel chromogenic reagent for the specific detection and enumeration of *Escherichia coli* in environmental samples. Can. J. Microbiol. **34**: 690–693.

Ratnam S, March S. B., Ahmed R., Bezanson G. S. and Kasatiya S. (1988): Characterization of *Escherichia coli* Serotype O157:H7. J. Clin. Microbiol. **26**: 2006-2012.

Restaino L., Frampton E.W. and Lyon R.H. (1990): Use of chromogenic substrate 5-bromo-4-chloro-3-indolyl- β- D-glucuronide (X-GLUC) for enumeration of *Escherichia coli* in 24 hours from ground beef. J. Food Prot., **53**: 508–510.

Ordering Information

Product	Cat. No.	Pack size	Other pack sizes available
Chromocult® TBX (Tryptone Bile X-glucuronide) Agar acc. ISO 16649	1.16122.0500	500 g	
ReadyPlate™ CHROM TBX Agar ISO 16649	1.46326.0020	20 x 90 mm	100 x 90 mm
GranuCult™ MMGA (Mineral Modified Glutamate) Agar acc. ISO 16649	1.09045.0500	500 g	

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