

## TBX Agar

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

### DESCRIPTION

TBX Agar is a chromogenic selective medium used for the isolation and identification of *Escherichia coli* in foods, according to ISO 16649-1, -2 and -3.

### TYPICAL FORMULA

	(g/l)
Enzymatic Digest of Casein	20.0
Bile Salts No. 3	1.5
X-Glucuronide	0.075
Agar	15.0
Final pH 7.2 ± 0.2 at 25°C	

### METHOD PRINCIPLE

Enzymatic digest of casein provides amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Bile salts act as selective agent inhibiting most Gram-positive bacteria.

5-bromo-4-chloro-3-indolyl- $\beta$ -D-glucuronide (BCIG) is the chromogenic substrate cleaved by the enzyme  $\beta$ -glucuronidase of *E. coli*. Agar is the solidifying agent.

### 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.

Medium in bottles Melt the content of the bottle in a water bath at 100°C (loosing the cap partially removed) 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.

### TEST 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.

### INTERPRETING RESULTS

After incubation observe the color of the colonies and interpret the results as indicated in the ID table.

Count typical CFU in all plates containing 15-300 colonies in total (typical and non-typical).

#### ID Table.

Microorganism	Typical colony color
$\beta$ -glucuronidase-positive <i>Escherichia coli</i>	Blue to blue-green
$\beta$ -glucuronidase-negative bacteria (if not inhibited)	White to green-beige

### APPEARANCE

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

Prepared medium: slightly opalescent, colorless to light beige.

### STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles and prepared plates at 2-8°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

**SHELF LIFE**

Dehydrated medium: 2 years.  
 Medium in bottles: 1 year.  
 Ready-to-use plates: 4 months.

**QUALITY CONTROL**

Plates are inoculated with the microbial strains indicated in the QC table.  
 Inoculum for productivity: 50-100 CFU.  
 Inoculum for selectivity: 10<sup>4</sup>-10<sup>6</sup> CFU.  
 Incubation conditions: aerobically at 44 ± 1°C for 18-24 hours.

**QC Table.**

Microorganism		Growth	Specification
<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

**WARNING AND PRECAUTIONS**

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for professional use only and must be used by properly trained operators.

**DISPOSAL OF WASTE**

Disposal of waste must be carried out according to national and local regulations in force.









**BIBLIOGRAPHY**

- ISO 16649 (2001) 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 – Part 2: Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl- $\beta$ -D-glucuronide – Part 3: Most probable number technique.
- Ogden I.D., and A.J. Watt (1991) An evaluation of fluorogenic and chromogenic assays for the direct enumeration of *E. coli*. Letters in App. Microbiol. 13:212-215.
- Delisle G.L., and A. Ley (1989) Rapid detection of *E. coli* in urine samples by a new chromogenic  $\beta$ -Dglucuronidase assay. J. Clin. Microbiol. 27:778-779.

**PRESENTATION**

		Contents	Ref.
TBX Agar	90 mm ready-to-use plates	20 plates	10522
TBX Agar	60 mm ready-to-use plates	20 plates	163652
TBX Agar	Bottles	6 x 100 ml bottles	481170
TBX Agar	Dehydrated medium	500 g of powder	610224
TBX Agar	Dehydrated medium	100 g of powder	620224

**TABLE OF SYMBOLS**

<b>LOT</b> Batch code	 Keep away from sunlight	 Manufacturer	 Use by	 Fragile, handle with care
<b>REF</b> Catalogue number	 Temperature limitation	 Contains sufficient for <n> tests	 Caution, consult Instruction For Use	 Do not reuse

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