



## Tryptone Bile Glucuronic Agar (TBX Agar)

M1591

Tryptone Bile Glucuronic Agar is selective agar for the detection and enumeration of *Escherichia coli* in foodstuffs and animal feed and water.

### Composition\*\*

Ingredients	Gms / Litre
Bile salt mixture	1.500
Enzymatic digest of casein	20.000
X-β-D-glucuronic acid	0.075
Dimethyl sulfoxide	3.000
Agar	15.000
Final pH ( at 25°C)	7.2±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 39.6 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C, mix gently and pour in sterile Petri plates.

### Principle And Interpretation

The formulation of Tryptone Bile Glucuronic Agar is in accordance with ISO 16649-2 (4). Tryptone Bile Glucuronic Agar contains the enzyme β-D- glucorinodase which differentiates most *E.coli* species from other coliforms. *E.coli* absorbs the chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (1).The enzyme β-glucorinodase splits the bond between the chromophere 5-bromo-4-chloro-3-indolyl and the β-D-glucuronide. *E.coli* colonies are blue green coloured (2,3). Growth of accompanying gram positive flora is largely inhibited by the use of bile salts and the high incubation temperature of 44°C.

### Quality Control

#### Appearance

Cream to yellow coloured homogeneous free flowing powder

#### Gelling

Firm, comparable with 1.5% Agar gel

#### Colour and Clarity of prepared medium

Yellow coloured clear to slightly opalescent gel forms in Petri plates

#### Reaction

Reaction of 3.66% w/v aqueous solution at 25°C. pH : 7.2±0.2

#### pH

7.00-7.40

#### Cultural Response

Cultural characteristics observed after an incubation at 44°C for 18- 24 hours.

#### Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
<b>Cultural Response</b> <i>Citrobacter freundii</i> ATCC 8090	>=10 <sup>3</sup>	inhibited	0%	
<i>Escherichia coli</i> ATCC 25922	50-100	luxuriant	>=50%	blue-green
<i>Enterococcus faecalis</i> ATCC 29212	>=10 <sup>3</sup>	inhibited	0%	

### Storage and Shelf Life

Store dehydrated and prepared medium at 2-8°C. Use before expiry date on the label.

## Reference

1. Frampton E W, Restaino L, Blaszkowski L. 1988. Evaluation of  $\beta$ -glucuronidase substrate 5-bromo-4-chloro-3-indolyl-B-D-glucuronide (X-GLUC) in a 24 hour direct plating method for Escherichia coli. J. Food Protection 51:402-404.
2. Killian M. and Bolow P 1976 Rapid diagnosis of Enterobacteriaceae I. Detection of bacterial glycosidases. Acta Rattol. Microbiol Scand Sect B 84:245-251.
3. Ley A N, Bowers R J, Wolfe S 1988 Indocyl -B-D-glucuronide, a novel chromogenic coli reagent for the detection and enumeration of Escherichia coli in environmental samples. Canadian Journal of Microbiology 34:690-693.
4. International Standard ISO 16649-2: 1999. Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of presumptive Escherichia coli; Part 2: Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl- $\beta$ -D-glucuronic acid.

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