



Technical Data

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 chromophore 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
Cultural Response				
<i>Citrobacter freundii</i> ATCC 8090	>=10 ³	inhibited	0%	
<i>Escherichia coli</i> ATCC 25922	50-100	luxuriant	>=50%	blue-green
<i>Enterococcus faecalis</i> ATCC 29212	>=10 ³	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 β -glucuronidase substrate 5-bromo-4-chloro-3-indolyl- β -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 - β -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- β -D-glucuronic acid.

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