

Harlequin™ Tryptone Bile Glucuronide Agar (TBX) (NCM1001)

Intended use

Harlequin™ Tryptone Bile Glucuronide Agar (TBX) is used for the enumeration of *E. coli* in food and animal feed and is not intended for use in the diagnosis of disease or other conditions in humans.

Description

Tryptone Bile Glucuronide Agar is recommended by ISO 16649-1:2001, ISO 16649-2:2001 and ISO 16649-3:2015 for enumeration of β -glucuronidase positive *Escherichia coli* in products intended for human consumption or for the feeding of animals. The medium contains tryptone as a source of nitrogen and amino acids, bile salts act as a selective against Gram-positive organisms and agar is the gelling agent. 5-bromo-4-chloro-3-indolyl- β -D-glucuronide (X-glucuronide) is a chromogen for the detection of the β -D-glucuronidase enzyme which is present in most *E.coli** but not other coliforms. The intracellular enzyme present in *E.coli* is able to break the bond between the substrate and the chromophore which dimerizes in the presence of oxygen to form an insoluble colored compound within the cell. The accumulation of the colored chromophore results in blue colonies on the agar.

*96-97% of *E.coli* are β -D-glucuronidase positive. A notable exception is *E.coli* O157:H7

Typical Formulation

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

Final pH: 7.2 $\pm$ 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. Refer to SDS

Preparation

1. Suspend 36.5 grams of the medium in 1 liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C.

Test Procedure

- For the enumeration of β -glucuronidase-positive *Escherichia coli* – Part 1: Colony-count technique at 44°C using membranes and 5-bromo-4-chloro-3-indolyl β -D-glucuronide – Refer to ISO 16649-1:2001
- For the enumeration of β -glucuronidase-positive *Escherichia coli*. Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl- β -D-glucuronide – Refer to ISO 16649-2:2001
- For the enumeration of beta-glucuronidase-positive *Escherichia coli*. Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl- β -D-glucuronide – Refer to ISO 16649-3:2015

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared clear to moderately hazy and yellow.

Expected Cultural Response: Cultural response on Harlequin™ Tryptone Bile X-Glucuronide (TBX) Medium at 44 $\pm$ 1°C and examined for growth at 18 – 24 hours incubation.



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Technical Specification Sheet



<u>MICROORGANISM</u>	<u>Culture ID</u>	<u>APPROX. INOCULUM (CFU)</u>	<u>EXPECTED RESULTS</u>	
			<u>Growth</u>	<u>Reaction</u>
<i>Enterobacter aerogenes</i>	ATCC 13048	4 Quad Streak	Growth	White
<i>Escherichia coli</i>	ATCC 25922	80-120	>50%	Blue
<i>Escherichia coli</i>	ATCC 35218	80-120	>50%	Blue
<i>Escherichia coli</i>	ATCC 11775	80-120	>50%	Blue
<i>Escherichia coli</i>	ATCC 8739	80-120	>50%	Blue
<i>Escherichia coli</i>	NCTC 13216	80-120	>50%	Blue
<i>Citrobacter freundii</i>	ATCC 43864	4 Quad Streak	Growth	Cream/White
<i>Pseudomonas</i>	ATCC 27853	4 Quad Streak	Growth	--
<i>Enterococcus faecalis</i>	ATCC 29212	> 10 ⁴	Complete Inhibition	--
<i>Enterococcus faecalis</i>	ATCC 19433	> 10 ⁴	Complete Inhibition	--

The organisms listed are the minimum that should be used for quality control testing.

Results

96-97% of *E.coli* are β -D-glucuronidase positive showing a blue color.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedures

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow.

Storage

Store dehydrated culture media at 2-30°C away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. ISO 16649-1:2001 – Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of β -glucuronidase-positive *Escherichia coli* – Part 1: Colony-count technique at 44°C using membranes and 5-bromo-4-chloro-3-indolyl β -D-glucuronide.
2. ISO 16649-2:2001 - Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of β -glucuronidase-positive *Escherichia coli*. Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl- β -D-glucuronide.
3. ISO 16649-3:2015 - Microbiology of the food chain. Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli*. Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl- β -D-glucuronide.



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