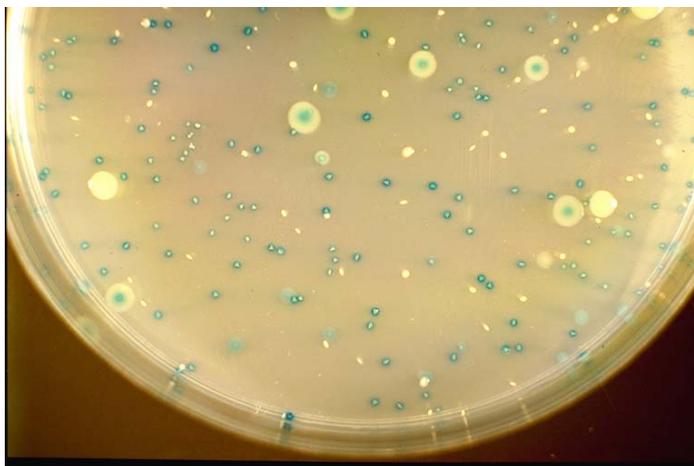


**TRYPTONE BILE X-GLUC (TBX) AGAR**  
Powdered and ready to use chromogenic medium  
for the detection of  $\beta$ -glucuronidase *positive E. coli* in foodstuff.



TBX Agar: *E.coli* (blue colonies ) and *E.aerogenes* (colourless colonies)

**TYPICAL FORMULA (g/l)**

Tryptone (Enzymatic digest of casein)	20
Bile Salts No. 3	1,5
Agar	14
5-bromo-4-chloro-3-indoyl-beta-D-glucuronide (X-GLUC) <sup>a</sup>	0,075

Note

a: cyclohexammonium salt

Final pH 7.2  $\pm$  0.2

**DIRECTIONS FOR POWDERED MEDIUM**

Suspend 35,6g in 1000ml of cold distilled water. Heat to boiling with agitation until complete dissolution and autoclave at 121°C for 15 minutes. Cool to 44-47°C and distribute into sterile Petri dishes.

**DIRECTIONS FOR READY TO USE MEDIUM IN FLASKS**

Dissolve the contents of the bottle in a temperature controlled water batch (100°C). Cool to 44-47°C, mix well and distribute into sterile Petri dishes

**DESCRIPTION**

Tryptone Bile X-GLUC (TBX) Agar is a selective and chromogenic medium for the enumeration of  $\beta$ -glucuronidase positive *Escherichia coli* in food and animal feeding stuffs. It is prepared according to the formulation given by ISO 16649-1 and ISO 16649-1. TBX Agar can be used with an incubation temperature of 44°C both with poured plated method (ISO 16649-2) and with membrane filtration technique (ISO 16649-1)

The medium contains bile salts for the complete inhibition of Gram-positive bacteria and X-GLUC (5-bromo-4-chloro-3 indoyl  $\beta$ -D-glucuronide) for the detection of  $\beta$ -glucuronidase enzyme.

Among the *Enterobacteriaceae* only *E. coli*, together with some strains of *Salmonella* and *Shigella*, is one of the few  $\beta$ -glucuronidase positive species, so cultivates on the plates with blue colonies.  $\beta$ -glucuronidase negative bacteria grow with colourless colonies.

**TECHNIQUE****POURED PLATE TECHNIQUE**

- Transfer 1ml of the test sample in duplicate, into two sterile Petri dishes if liquid, or 1ml of the initial suspension ( $10^{-1}$ ), in the case of other products. Repeat the procedure with further decimal dilutions if necessary.

- Within 15 minutes, pour into each Petri dish about 15ml of TBX Agar pre-cooled to 44-47°C.
- Mix well the inoculum with the medium. Invert the inoculated dishes and incubate at 44°C for 18-24 hours. In cases where stressed colonies are suspected incubate for 4 hours at 37°C before incubation at 44°. Do not incubate over 45°C.
- Count as  $\beta$ -glucuronidase positive *E.coli* the blue colonies in the plates with less than 150 typical colonies and less than 300 total colonies (typical and atypical)
- Report the results as UFC/g considering the "dilution factor" and according to the recommendations of ISO norms.

**MEMBRANE FILTRATION TECHNIQUE**

- Transfer 2 sterile membranes onto the surface of 2 plates of Minerals Modified Glutamate Medium (MMGM- REF 401737) .
- Transfer in the centre of the membrane 1 ml of the sample or 1 ml of the initial suspension and spread the inoculum on the surface of the membrane. Repeat the procedure with further decimal dilutions if necessary.
- Leave the plates at room temperature for 15 minutes in order the medium adsorbs the liquid sample.
- Incubate the plates for 4 h  $\pm$  1 h at 37°C.
- After this resuscitation step transfer the membranes onto TBX Agar plates and incubate at 44°C for 18-24 hours. Do not incubate over 45°C.
- Count as  $\beta$ -glucuronidase positive *E.coli* the blue colonies in the plates with less than 150 typical colonies and less than 300 total colonies (typical and atypical)
- Report the results as UFC/g considering according to the recommendations of ISO norms.

**USER QUALITY ASSURANCE (44°C - 24 hrs)**

Productivity control: *E.coli* ATCC 25922: growth, blue-green colonies

Specificity control: *S.typhimurium* ATCC 27853: growth, green-beige colonies

Selectivity control: *E.faecalis* ATCC 19433: inhibited

**STORAGE**

Store the dehydrated culture medium at 2-8°C in the original bottle, tightly closed, away from bright light and use before the expiry date on the label. The expiry date should be applied to both the open and on-open bottles stored as recommended.

Use the plates or flasks prepared in Laboratory within 7 days.

Store the ready to use flasks at 2-8°C and use before the expiry date on the label

**WARNINGS**

Powdered TBX Agar is not classified as dangerous according to the applicable regulations but it contains bile salts at a concentration higher than 1% than it requires the availability of Material Safety Data Sheet; please consult MSDS before the use and do not inhale and do not ingest the product.

Ready to use TBX Agar is not classified as dangerous according to the applicable regulations.

**REFERENCES**

- ISO 16649-2 (2001). Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive *E.coli*- part 2: colony count technique at 44°C using 5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid.
- ISO 16649-1 (2001). Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive *E.coli*- part 2: colony count technique at 44°C using membranes and 5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid.

**PACKAGING**

<b>4021561</b>	<b>Tryptone Bile X-GLUC (TBX) Agar</b>	<b>100g (2,8 l)</b>
<b>4021562</b>	<b>Tryptone Bile X-GLUC (TBX) Agar</b>	<b>500g (14 l)</b>
<b>5121562</b>	<b>Tryptone Bile X-GLUC (TBX) Agar</b>	<b>6 x 100 ml ready to use flasks</b>
<b>542156</b>	<b>Tryptone Bile X-GLUC (TBX) Agar</b>	<b>20 ready to use plates Ø 90 mm</b>
<b>502156P</b>	<b>Tryptone Bile X-GLUC (TBX) Agar</b>	<b>5 ready to use plates Ø 140 mm</b>
<b>492156</b>	<b>Tryptone Bile X-GLUC (TBX) Agar</b>	<b>30 ready to use plates Ø 55 mm</b>