TECHNICAL DATA SHEET

TRYPTONE-BILE-X-GLUCURONATE (TBX) AGAR

ENUMERATION OF *ESCHERICHIA COLI* β-D-GLUCURONIDASE POSITIVE

1 INTENDED USE

TBX Agar is a selective medium for the enumeration of β-D-glucuronidase-positive *Escherichia coli* in food products and environmental samples of production area. The result is obtained directly by counting characteristic colonies after only 24 hours of incubation and no confirmation step is required.

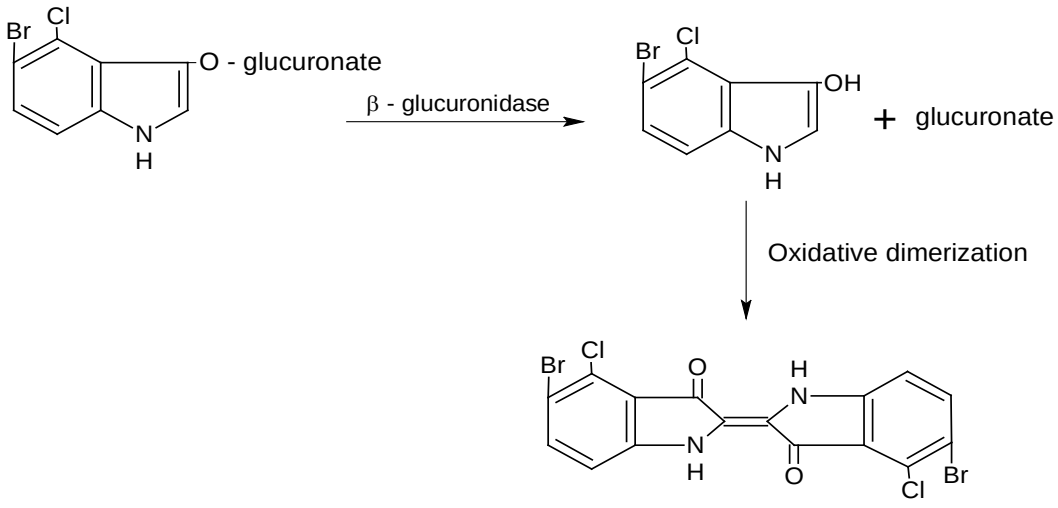
The typical composition corresponds to that defined in the standards NF ISO 16649-1, NF ISO 16649-2 and NF EN ISO 16649-3.

2 HISTORY

In 1949, Buehler *et al*. were the first to report the presence of a β-D-glucuronidase in *Escherichia coli*. Since then, most studies have shown that 94 to 97% of *Escherichia coli* of human or environmental origin possess β-D-glucuronidase. This enzyme could also be detected in *Citrobacter, Enterobacter, Klebsiella, Salmonella, Shigella* and *Yersinia*, but its presence involves only a limited number of strains of each of these species.. β-D-glucuronidase can thus be considered to be a valid marker for the detection of *Escherichia coli* in food products and in water samples. In 1990, Restaino successfully used a new chromogenic substrate : BCIG. When incorporated into tergitol agar, it enables *Escherichia coli* in meat products to be counted in 24 hours. In contrast, TBX agar has been specifically formulated without using tergitol as a selective agent. In its place are substituted bile salts which confer similar selective properties.

3 PRINCIPLES

BCIG (5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid) is a chromogenic substrate. Most *Escherichia coli* strains possess a β-D-glucuronidase that cleaves BCIG, causing the colonies to become blue according to the following reaction mechanism :



Blue precipitate of dichloro-dibromo indigo

It is important to note that not all *Escherichia coli* possess β-D-glucuronidase, in particular enterohemorrhagic serotype 0157:H7, which forms white colonies.
Bile salts inhibit the growth of Gram-positive bacteria and favor the recovery of *Escherichia coli.*

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain the optimal performance.

For 1 liter of media :

- Tryptone ..... 20,0 g
- Bile salts n°3 ..... 1,5 g
- BCIG (5-bromo-4-chloro-3-indolyl β-D-glucuronate)..... 75,0 mg
- Bacteriological agar..... 9,0 g

pH of the ready-to-use media at 25 °C : 7,2 ± 0,2.

5 PREPARATION

Preparation from dehydrated media :

- Dissolve 30,6 g of dehydrated media (BK146) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation for the minimum amount of time to achieve complete dissolution.
- Dispense in tubes or vials.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool and maintain the media in a molten state at 47-50 °C.

✓ **Reconstitution : 30,6 g/L**

✓ **Sterilization : 15 min at 121 °C**

Use from ready-to-melt media :

- If the media was prepared advance as above, or when using the ready-to-melt agar (BM069 or BM171), heat the vials for the least amount of time to achieve total liquefaction.
- Cool and maintain in a molten state at 47-50°C.

6 INSTRUCTIONS FOR USE

Enumeration on membrane (NF ISO 16649-1)

- Inoculate a membrane with 1 mL of inoculum and set on the surface of Modified Glutamate agar.
- Incubate for 4 ± 0.25 h at 37 ± 1°C.
- Peel off the membrane from the previous plate and place it gently on the surface of solidified TBX agar. .
- Incubate for 20 to 24 hours at 44 ±1 °C.

✓ **Inoculation : Deposit membrane**

✓ **Incubation : 20 h to 24 h at 44 ± 1 °C**

Pour plate inoculation (NF EN ISO 16649-2)

- Transfer 1 mL of the initial suspension and its serial dilutions to successive Petri plates.
- Pour roughly 15 mL of molten media held at 44-47 °C, per plate.
- Mix well and let solidify on a cold, flat surface. .
- Incubate at 44 ± 1 °C for 18 to 24 hours maximum.

✓ **Inoculation : 1 mL pour plate**

✓ **Incubation : 18 h to 24 h at 44 ± 1 °C**

NOTE

If the presence of stressed microorganisms is suspected, incubate first for 4 hours at 37°C, then for 18 to 24 hours at 44°C.

Enumeration by MPN method (NF EN ISO 16649-3)

- Pour the media into sterile Petri plates and solidify on a cold, flat surface.
- Dry the plates in an incubator, covers partially removed.
- Re-inoculate each tube of Modified glutamate broth (BK186) presumptive positives onto the TBX media as prepared above.
- Incubate at 44 ± 1°C for 20 to 24 hours.

✓ **Inoculation : Streaking on surface**

✓ **Incubation : 20 to 24 h at 44 °C**

7 RESULTS

Characteristic colonies are blue to blue-green.

Proceed with colony counts using plates containing less than 150 characteristic colonies and less than 300 total (characteristic and non-characteristic) colonies.

See ANNEX 1 : PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated media : cream-white powder, free-flowing and homogeneous.
Prepared media : whitish agar.

Typical culture response after 21 hours of incubation at 44 °C (NF EN ISO 11133) :

Microorganisms		Growth (Productivity Ratio : P_R)	Characteristics
Escherichia coli	WDCM 00013	$P_R \geq 50\%$	Blue colonies
Escherichia coli	WDCM 00202	$P_R \geq 50\%$	Blue colonies
Citrobacter freundii	WDCM 00006	Not inhibited, score 2	White colonies
Pseudomonas aeruginosa	WDCM 00025	Not inhibited, score 2	White to grey-beige colonies
Enterococcus faecalis	WDCM 00087	Inhibited, score 0	-

9 STORAGE / SHELF LIFE

Dehydrated media : 2-20 °C.
Ready-to-melt media in vials : 2-8 °C, shielded from light.
The expiration dates are indicated on the labels.

Prepared media in vials (*) : 180 days at 2-8 °C shielded from light..
Prepared media in plates (*) : 15 days at 2-8 °C, shielded from light..
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated media :
100 g vial..... BK146HM
500 g vial..... BK146HA

Ready-to-melt media :
10 x 100 mL vials..... BM06908
10 x 200 mL vials..... BM17108

11 BIBLIOGRAPHY

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12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

Document code : AGAR TBX_ENv11
Creation date : 01-2003
Updated : 09-2018
Origin of revision : Update of instruction for use, bibliography.

ANNEX 1 : PHOTO SUPPORT

TBX Agar

Enumeration of *Escherichia coli* β-D-glucuronidase positive.

Results :

Growth obtained after 24 hours of incubation at 44 °C (pour plates).

***Escherichia coli* β-glucuronidase positive**

Characteristic colony :
Blue to blue-green color