

**PRODUCT CODE: 416220**

## **TBX Agar (ISO 16649-2,3:2001)(Dehydrated Culture Media) for microbiology**

### **Preparation**

Suspend 36.5 g of the powder in 1 L of distilled water and heat to boiling with continuous stirring until total dissolution. Dispense into suitable containers and sterilize in the autoclave at 121°C for 15 minutes. Cool to 45-50°C, mix well and dispense into plates. The prepared medium should be stored at 8-15°C.

The colour is amber, slightly opalescent. The dehydrated medium should be homogeneous, free-flowing and beige in colour. If there are any physical changes, discard the medium.

### **Uses**

*Escherichia coli* is the only coliform that possesses  $\beta$ -D-glucuronidase and can be easily differentiated from other coliforms that do not show this enzymatic activity. There are some strains of *E. coli* (less than 3-4% of the total population) that are  $\beta$ -D-glucuronidase negative.

*E. coli* absorbs the chromogenic substrate (X- $\beta$ -D-glucuronide) and the bacterial enzyme  $\beta$ -D-glucuronidase splits the bond between the chromophoric X-fraction and the  $\beta$ -D-glucuronide.

The free X-fraction dyes the *E. coli* cells and produces a blue-green colony.

The high content in bile salts of the medium inhibits the growth of accompanying Gram positive bacteria and the high incubation temperature (44°C) inhibits Gram negative bacteria other than *E. coli*.

### **Technique**

#### 1. Direct inoculation (Pour plate technique)

Transfer 1 mL of test sample to a sterile Petri dish aseptically and repeat the procedure with further dilutions. Inoculate two plates per dilution. Pour 15 mL of melted and cooled (44-47°C) TBX Agar into each Petri dish. Mix carefully and allow the mixture to solidify. The time between the distribution of the inoculum and pouring the medium should not exceed 15 minutes.

Invert the inoculated plates and incubate them at 44°C for 18-24 hours. If the presence of stressed cells is suspected incubate for an initial period of 4 hours at 37°C and then raise the incubation temperature to 44°C. The total incubation time should not exceed 24 hours and the incubation temperature should not exceed 45°C.

#### 2. Membrane incubation (Resuscitation technique)

No special membranes are recommended. Any sterile and non-inhibitive membrane made of cellulose acetate or mixed esters of cellulose, with 0.45  $\mu$ m to 1.2  $\mu$ m pore size and 85 mm diameter can be used.

##### 2.1. Resuscitation

Aseptically place a membrane on the dried surface of each of two plates of Mineral-Modified-Glutamate Agar (Art. No. 416895.1210) with care to avoid trapping air bubbles. Add 1 mL of the test sample to the centre of each membrane and spread the inoculum evenly over the whole membrane surface. Repeat the procedure for each dilution of the sample.

Leave the inoculated plates at room temperature for 15 minutes until the inoculum has soaked into the agar. Incubate the plates at 37°C for 4 ± 1 hours.

#### 2.2. Transfer to the selective medium

After the resuscitation period, transfer the membranes from the resuscitation medium to the plates of TBX Agar using sterile forceps, taking care to avoid trapping air bubbles beneath the membrane. Do not touch nor disturb the membrane surface. Incubate the plates for 18-24 hours at 44°C (and not more than 45°C).

#### 3. Results

The β-D-glucuronidase-positive *Escherichia coli* produces blue colonies (Blue-green). Some strains (3-4% of the total population) of *E. coli* lack the glucuronidase enzyme and produce colourless colonies. Some stressed cells of *E. coli* are unable to grow at 44°C and produces no colonies.

### Composition

See in Data Sheet (TDS).

### Microbiological Test

Incubation temperature: 44 ± 1° C Incubation time: 21 ± 3 h

Inoculum: Practical range 100±20 CFU. Min. 50 CFU (Productivity) / 104-106 CFU (Selectivity) / 103-104CFU (Specificity) according to ISO 11133:2014.

Microorganism	Growth	Colony Colour
<i>Enterococcus faecalis</i> ATCC® 19433	Total inhibition	Selectivity
<i>Escherichia coli</i> ATCC® 25922	Productivity > 0.50	Blue colonies
<i>Escherichia coli</i> ATCC® 11775	Productivity > 0.50	Blue colonies
<i>Citrobacter freundii</i> ATCC® 43864	Good (Specificity)	Colorless colonies
<i>Pseudomonas aeruginosa</i> ATCC® 27853	Good (Specificity)	Colorless colonies

### Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).

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