



ChromoCult® TBX Agar

Selective agar for the detection and enumeration of
Escherichia coli in foodstuffs, animal feed and water.



ChromoCult® TBX (Tryptone Bile X-glucuronide) Agar



Ord. No. 1.16122.0500 (500g)

Selective agar for the detection and enumeration of *Escherichia coli* in foodstuffs, animal feed and water. The medium complies with the recommendations of ISO 16649-1+2, 2000.

Mode of action

The presence of the enzyme β -D-glucuronidase differentiates most *E.coli* ssp. from other coliforms. *E.coli* absorbs the chromogenic substrate 5-bromo-4-chloro-3-indolyl- β -D-glucuronide (X- β -D-glucuronide). The enzyme β -glucuronidase splits the bond between the chromophore 5-bromo-4-chloro-3-indolyl- and the β -D-glucuronide. *E.coli* colonies are coloured blue-green.

Growth of accompanying Gram-positive flora is largely inhibited by the use of bile salts and the high incubation temperature of 44 °C.

Typical composition (g/Litre)

Peptone 20.0; bile salts No. 3 1.5; X- β -D-glucuronide 0.075; agar-agar 10.0.

Preparation

Suspend 31.6 g in 1 litre of demin. water by heating in a boiling water bath or in flowing steam until the medium is completely dissolved. Autoclave at 121 °C for 15 min.

Cool to 45-50 °C in a water bath, mix gently and pour 15 ml in sterile Petridishes. pH: 7.2 \pm 0.2 at 25 °C. The prepared medium is clear and yellowish. If stored at +2 to +8 °C and protected from light plates or medium in bottles are stable for 4 weeks.

Experimental procedure

The pour plate or membrane filtration technique can be used to inoculate the medium.

Pour plate technique: Pipette 1 ml of a homogenate or appropriate 10-fold dilution into a sterile Petridish, add 15 ml of the medium (cooled to 45-50 °C) and mix gently.

Fresh or raw samples: Plates are incubated at 44 °C for 18-24 h aerobically.

Membrane filtration technique: Filter an aliquot of a liquid sample through a Cellulose-mix-ester Membrane e.g. Gelman GN 6.

Fresh or raw samples: Transfer the membrane-filter to ChromoCult® TBX agar and incubate at 44 °C for 18-24 h.

ISO 16649-1 membrane filtration technique: For the recovery of sublethally injured *E.coli*.

Resuscitation step: The membrane filter is transferred to Mineral modified Glutamat Agar (MMGA) and incubate at 37 °C or 30 °C for 4 h. After this resuscitation step transfer the membrane-filter to ChromoCult® TBX Agar and incubate at 44 °C for another 18-20 h.

ISO 16649-2 pour plate technique: Pipette 1 ml of a homogenate or appropriate 10-fold dilution into a sterile Petridish, add 15 ml of the medium (cooled to 45-50 °C) and mix gently.

Resuscitation step: For the recovery of sublethally injured *E.coli*, plates are incubated at 37 °C or 30 °C for 4 h. After this resuscitation step incubation is continued at 44 °C for another 18-20 h.

Results

E.coli colonies are blue-green (X- β -D-glucuronide reaction).

Attention: β -Glucuronidase-negative *E.coli* strains (3-4%) form colourless colonies, e.g. *E.coli* 0157, or they cannot grow at elevated temperature of 44 °C, e.g. *E. coli* 0157: H7.





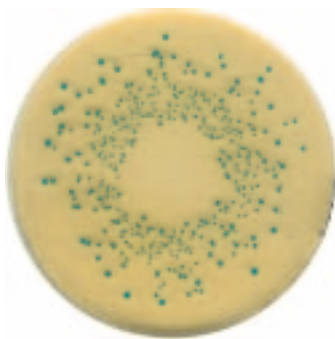
Colour makes the difference.

Quality control using the spiral plate method

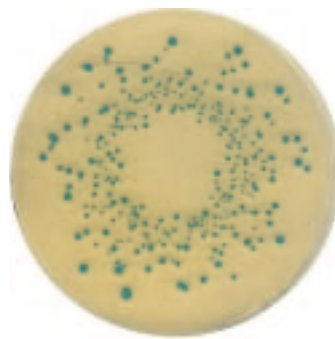
| Test strains | Inoculum (cfu/ml) | Colony colour | Recovery rate |
|-------------------------------------|-------------------|---------------|---------------|
| Escherichia coli DSMZ 502 | 10^3 - 10^5 | blue-green | $\geq 70\%$ |
| Citrobacter freundii ATCC 8090 | $\geq 10^5$ | - | $\leq 0,01\%$ |
| Enterococcus faecalis ATCC 19433 | $\geq 10^5$ | - | $\leq 0,01\%$ |

Literature

International Standard ISO 16649-1+2: Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of presumptive Escherichia coli
Part 1: Colony-count technique at 44 °C using membranes and 5-bromo-4-chloro-3-indolyl- β -D-glucuronide
Part 2: Colony-count technique at 44 °C using 5-bromo 4-chloro-3-indolyl- β -D-glucoronic acid (2001).



Escherichia coli
ATCC 25922



Escherichia coli
DSMZ 502

Ordering Information

| Product | Merck Ord. No. | Pack size |
|--|----------------|-----------|
| ChromoCult® TBX (Tryptone Bile X-glucuronide) Agar | 1.16122.0500 | 500 g |
| GN6 Cellulose-Mischester-Membranfilter | PALL | |
| Mineral modified Glutamat Agar (MMGA) | 1.09045.0500 | 500 g |

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