92435 TBX Agar (Tryptone Bile X-glucuronide Agar)

Chromogenic Agar for the detection and enumeration of *Escherichia coli* in foodstuffs, animal food and water without further confirmation. *E. coli* colonies are coloured blue-green.

Composition:

Ingredients	Grams/Litre
Peptone	20.0
Bile salts	1.5
X-ß-D-glucuronide	0.075
Agar	15.0

Final pH 7.2 +/- 0.2 at 25°C
Store prepared media below 8 °C, protected from direct light the plates are stable for 1 week. Store dehydrated powder in a dry place in tightly-sealed containers at 2-25 °C.

Directions:
Suspend 36.6 g in 1 liter distilled water. Heat 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 petri dishes.
Dilute food samples 1: 5 or 1:10 with 0.1% (w/v) sterile Peptone Water (70179) and homogenize in a blender or a stomacher.
Pour-plate method: For enumeration, give 1 ml of the homogenized sample material on a petri dish and dilute with 15 ml of the TBX Agar (cooled to 45-50 °C) and mix gently.
Spread-plate method: Pipette 0.5 ml or 1.0 ml of the homogenized food sample on to the plate and spread with sterile glass spreader.
Also the membrane filtration technique is applicable. An incubation of the filter in DEV Glutamate Broth (31435) with 15 g Agar (05040), for 4 hours at 30°C, increase the recovery rate.
Incubate the plates at 30°C for 4 hours and then at 44°C for 18 hours to save the sublethally injured *Escherichia coli*.

Principle and Interpretation:
Tryptone Bile Agar is for the detection of *Escherichia coli* in foodstuffs and water (1), where recovery of Escherichia coli is faster, more reliable and accurate. Most of the *Escherichia coli* strains can be differentiated from other coliforms by the presence of enzyme glucuronidase which is highly specific for *Escherichi coli* (2). The chromogenic agent X-glucuronide used in this medium helps to detect glucuronidase activity. *Escherichia coli* cells absorb x-glucuronide and the intracellular glucuronidase splits the bond between the chromophore and the glucuronide. The released chromophore gives coloration to the colonies. The peptone provides the essential growth nutrients to the organisms. Bile salts mixture inhibits gram-positive organisms.

Cultural characteristics after 18-24 hours at 44°C.

Organisms (ATCC)	Growth	Color of colony
Escherichia coli (25922)	+++	blue-green
Salmonella enteritidis (13076)	+++	colorless
Citrobacter freundii (8090)	-	-
Enterococcus faecalis (19433)	-	-
Staphylococcus aureus (25923)	-	-

References:
1. J.M. Anderson, A.C. Baird-Parker, 1975, J. Appl. Bact., 39:1 1 1
2. W. Hansen, E Yourassawsky, 1984, J. Clin. Microbiol., 20:1177
3. Draft International Standard ISO/DIS 16649-2, Microbiology of food and animal feeding stuffs, Horizontal method for the enumeration of presumptive Escherichia coli, Part 2: Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl-ß-D-glucoronic acid (1999)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.