

TTC Chapman Agar (Lactose Agar TTC with Tergitol 7)

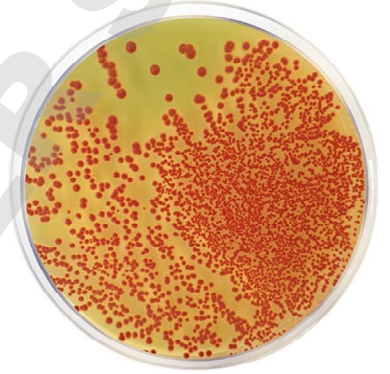
Cat. 1076

For the detection and enumeration of E.coli and coliforms in drinking water by membrane filtration technique.

Practical information

| Applications | Categories |
|-----------------------|------------------|
| Selective enumeration | Coliforms |
| Selective enumeration | Escherichia coli |
| Detection | Coliforms |
| Detection | Escherichia coli |

Industry: Water



Principles and uses

TTC Chapman Agar (Lactose Agar TTC with Tergitol 7) is a selective and differential medium used for the presumptive control of E.coli and coliforms in waters for human consumption by the membrane filtration technique.

Peptone and beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. Lactose is a fermentable carbohydrate providing carbon and energy. Sodium heptadecylsulfate (Tergitol 7) and TTC inhibit most Gram positive bacteria. Bromothymol blue is a pH indicator. Bacteriological agar is the solidifying agent.

Formula in g/L

| | | | |
|-----------------|------|--|-----|
| Bromthymol blue | 0,05 | Bacteriological agar | 15 |
| Beef extract | 5 | Lactose | 20 |
| Peptone | 10 | Sodium heptadecyl sulfate (Tergitol 7) | 0,1 |
| Yeast extract | 6 | | |

Preparation

Suspend 56,15 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 °C for 15 minutes. Cool to 45-50 °C and aseptically add 2,5 ml of TTC 1% Supplement (Cat. 6030), previously reconstituted in 5 ml of sterile distilled water, to 1 liter of the medium. Homogenize gently and dispense into Petri dishes. DO NOT OVERHEAT.

Instructions for use

For the detection and enumeration of Escherichia coli and coliform bacteria in water samples:

- Filter two samples of water over two different membranes and incubate on TTC Chapman Agar at 36±2 °C and 44±4 °C respectively for 21±3 hours.

Typical colonies have the appearance as follow:

- E. coli and Citrobacter spp present yellow colonies with orange-colored center.
 - Enterobacter spp forms red colored colonies and dark yellow with orange-colored center. The medium is yellow.
 - Klebsiella spp form red colored or yellow, but without center. The medium is yellow.
 - Lactose non fermentative bacteria grow with purple colonies and change the medium to blue.
- Klebsiella and Enterobacter species can also produce yellow-green colonies.

The results will always refer to counts per 100 ml of sample, considering if it has been necessary to make dilutions.

- Count as lactose-positive bacteria the colonies that present a yellow development of the medium under the membrane.
- Subculture the characteristic colonies obtained, in non-selective agar and Tryptophan Culture Broth (Cat. 1237).
- Carry out the oxidase test and incubate the tubes of Tryptophan Culture Broth at 44±0,5 °C for 21±3 hours.

- Indole production is determined by adding a few drops of Kovac's Reagent (Cat. 5205) to the incubated Tryptophan Culture Broth tubes. A positive test is indicated by the development of red color in the reagent layer.
- The colonies that are oxidase negative will be considered as coliform bacteria and the colonies that are negative oxidase and positive indol will be considered as E.coli.

Quality control

| Solubility | Appearance | Color of the dehydrated medium | Color of the prepared medium | Final pH (25°C) |
|------------|-------------|--------------------------------|------------------------------|-----------------|
| w/o rests | Fine powder | Greenish-beige | Green | 7,2±0,1 |

Microbiological test

According to ISO 11133:

Incubation conditions: (36±2 °C / 21±3 h). Inoculation conditions: Productivity quantitative (100±20, Min. 50 CFU) / Selectivity (10⁴-10⁶ CFU) / Specificity (10³-10⁴ CFU).

Reference media: Media batch TTC Chapman Agar already validated.

| Microorganisms | Specification | Characteristic reaction |
|-----------------------------------|----------------------|---|
| Enterococcus faecalis ATCC 19433 | Total inhibition (0) | |
| Escherichia coli ATCC 25922 | Good growth >70 % | Yellow in the central part below the membrane |
| Pseudomonas aeruginosa ATCC 27853 | | Red colonies, blue colour in the medium |
| Escherichia coli CECT 8296 | Good growth >70% | Yellow in the central part below the membrane |

Storage

Temp. Min.:2 °C
Temp. Max.:25 °C

Bibliography

Chapman G.H. 1946. A single culture medium for selective isolation of plasma coagulating staphylococci and for improved testing of chromogenesis (J. Bacteriol. 51: 409-410).

Tittsler R.P. and L.A. Sandholzer. 1936. The Use of Semi-Solid Agar for the detection of bacteria motility. (J. Bacteriol 31: 575-580)

ISO 9308-1:2000. Water quality. Detection and enumeration of Escherichia coli and coliform bacteria.PART.1. Membrane filtration method