

Lactose TTC Agar with Tergitol® 7

Selective differential medium for the detection and enumeration of *E. coli* und coliform bacteria in water using the membrane filtration method.

The medium complies with the recommendations of the ISO 9308-1 (1988) and the AFNOR norm NF 90-414 (1985) Water quality – Detection and enumeration of *E. coli* and coliform bacteria – Membrane filtration method.

Mode of Action

Degradation of lactose to acid is indicated by the pH indicator bromothymol blue, which changes the colour of the medium under the membrane to yellow. Selectivity is achieved by the use of sodium heptadecylsulfate (Tergitol®7) and 2,3,5-Triphenyltetrazoliumchloride (TTC) to inhibit most Gram-positive bacteria. TTC is also part of the differential system. The reduction of TTC by lactose-negative bacteria produces dark red colonies. Lactose-positive *E. coli* and coliform bacteria reduce TTC weakly; hence their colonies are yellow-orange.

Typical Composition (g/litre)

Lactose 20.0; peptone 10.0; yeast extract 6.0; meat extract 5.0; bromothymol blue 0.05; Tergitol®7 0.1; agar-agar 12.7.

Additive: TTC 0.025.

Preparation

Suspend 53.9 g in 1 litre of demin. water, dissolve and autoclave (121 °C, 15 min). Cool the medium in a water bath to 45-50 °C, add 5 ml of a sterile filtrated 0.05 % aqueous solution of TTC to 100 ml basal medium. Mix homogeneously and pour the medium into Petridishes. The agar layer should have a height of at least 5 mm.

pH: 7.2 ± 0.2 at 25 °C.

The prepared medium is clear and green.

The TTC-solution and the medium is stable for 4 week when stored at +2° - +8°C and protected from light.

Experimental Procedure

Detailed instructions on titer determination are contained in APHA: Standard Methods for the examination of Water and Wastewater (1998).

The type of membrane filter affects the performance of the medium. The best results were obtained using cellulose-nitrate filters, e.g. from Sartorius (order no. 13906-47-ACN).

After filtration the filter is transferred, under aseptic conditions, to the agar surface.

Incubation: 21 ± 3 hours at 36 ± 2 °C.

Evaluation

Lactose-positive bacteria produce yellow-orange colonies and under the membrane yellow-orange halos. The count of these typical colonies is considered to be presumptive coliform bacteria count.

Confirmation of coliform and *E. coli* count requires further subculture of typical colonies on a non selective agar (e.g. CASO agar) and Tryptophan broth, respectively.

Colonies that are oxidase negative are considered to be **coliform bacteria**. Coliform bacteria that form indole from tryptophane at 44 ± 0.5 °C within 21 ± 3 hours are considered to be ***E. coli***.

Literature

CHAPMAN, G.H. 1947. A superior culture medium for the enumeration and differentiation of coliforms. - *J. Bact.* **53**: 504 T (1947).

KULP, W., MASCOLI, C., TAVSHANJIAN, O. 1953. Use of tergitol-7 triphenyl tetrazolium chloride agar as the coliform confirmatory medium in routine sanitary water analysis. - *Am. J. Publ. Hlth.* **43**: 1111-1113 (1953).

POLLARD, A.L. 1946. A useful selective bactericidal property of Tergitol-7. *Science* 103: 758-759.AE.

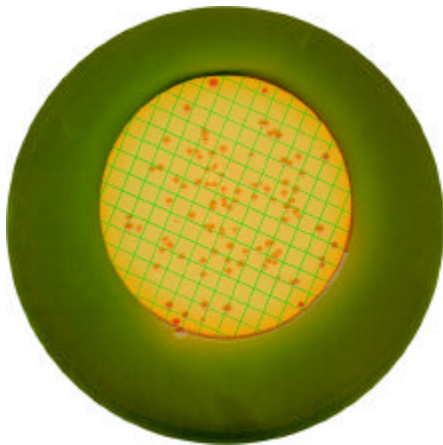
Ordering Information

Product	Merck Cat. No.	Pack size
Lactose TTC Agar with Tergitol® 7	1.07680.0500	500 g
2,3,5-Triphenyltetrazolium chloride	1.08380.0010	10 g
Bactident® Indole (dropper bottle)	1.11350.0001	1 x 30 ml
Bactident® Oxidase	1.13300.0001	50 test strips
CASO Agar (Casein Peptone Soymeal Peptone Agar)	1.05458.0500	500 g
CASO Agar (Casein Peptone Soymeal Peptone Agar)	1.05458.5000	5 kg
DEV-Tryptophan-Broth	1.10694.0500	500 g
KOVÁCS Indole Reagent	1.09293.0100	100 ml

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Quality control with the membrane filtration method

Test strains	Growth	Colour of medium (under membrane)	Colony colour	Oxidase	Indole (44 °C)
<i>Escherichia coli</i> ATCC 25922	+	yellow	yellow-orange	-	+
<i>Citrobacter freundii</i> ATCC 8090	+	yellow	yellow-orange	-	-
<i>Pseudomonas aeruginosa</i> ATCC 27853	+	blue	red	+	-
<i>Bacillus cereus</i> ATCC 11778	-				



Citrobacter freundii
ATCC 8090 von oben



Escherichia coli
ATCC 25922 von oben