

TTC 1% SUPPLEMENT

TTCS-00P-010

- **Principle**

TTC 1% Supplement is based on 2,3,5-triphenyltetrazolium chloride (TTC), a redox indicator widely used for the presumptive detection, isolation and enumeration of microorganisms such as *Escherichia coli* and other coliform bacteria in solid culture media. It is particularly suitable for microbiological analysis using the membrane filtration technique, especially in water testing and may also be used in milk testing.

TTC is colourless in its oxidized form and is reduced by metabolically active microorganisms to form formazan, an insoluble red pigment that accumulates within the cells. This reduction reaction produces characteristic colony colouration, facilitating the visual differentiation and presumptive identification of microbial growth on the supplemented medium.

When incorporated into an appropriate culture medium, TTC helps distinguish different groups of microorganisms according to their metabolic activity and colony appearance. Depending on the final TTC concentration, a slight inhibitory effect may occur in some Gram-positive microorganisms. The supplemented medium can therefore be used as a differential system for the presumptive detection and enumeration of coliform bacteria and *E. coli*, with subsequent confirmation by suitable microbiological and biochemical tests.

- **Regulatory compliance**

This product is manufactured under a quality management system in accordance with ISO 9001 and ISO 13485, and its formulation and quality control comply with applicable international standards, such as ISO 11133, where relevant.

- **Composition**

Ingredients	g/vial
TTC	0,05

- **Preparation**

Under aseptic conditions, reconstitute 1 vial with 5 ml of sterile distilled water and mix gently until complete dissolution.

Add 2,5 ml of the reconstituted supplement to 1000 ml of TTC Chapman Agar (cat. AGCH-001). Autoclaved and cool the complete medium to approximately 50 °C and dispense it into suitable sterile dishes.

When the TTC is required to be added to another media, refer to the specific instructions of the medium for the quantity of TTC 1% supplement that should be added.

- **Applications and use**

TTC 1% Supplement is intended for the preparation of differential culture media used for the presumptive detection and enumeration of *E. coli* and other coliform bacteria, particularly in water samples analysed by membrane filtration. It may also be used in microbiological testing of other sample types when TTC reduction is used as an indicator of microbial activity.

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 (AGCH-001) 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-coloured center.
- *Enterobacter spp* forms red coloured colonies and dark yellow with orange-colored center. The medium is yellow.
- *Klebsiella spp* form red coloured 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.
- Carry out the oxidase test and incubate the tubes of Tryptophan Culture Broth at $44 \pm 0,5$ °C for 21 ± 3 hours.
- 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**

Colour	White-yellowish
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- **Microbiological control**

For microbiological performance testing, inoculate using a practical range of 100 ± 20 CFU, with a minimum of 50 CFU for productivity testing and 10^4 – 10^6 CFU for selectivity testing.

Incubate under aerobic conditions at 36 ± 2 °C and evaluate the results after 21 ± 3 hours.

Microorganism	ATCC	WDCM	Growth	Recovery	Colonies
<i>Enterococcus faecalis</i>	19433	-	Inhibited	-	-
<i>Escherichia coli</i>	25922	00013	Good	≥ 70%	Yellow-orange under MF
<i>Ps. aeruginosa</i>	27853	-	Good	-	Red with blue center
<i>E. coli</i>	13167 (NCTC)	00179	Good	≥ 70%	Yellow-orange under MF.

- **Storage and shelf life**

Shelf life: 49 months. The declared expiry is valid only when stored as per the conditions mentioned on the label (Temp. Min.:2 °C Temp. Max.:8 °C).

- **Sterility control**

Sterility is verified by incubation for 48 hours at 30–35 °C and 48 hours at 20-25°C. No microbial growth shall be observed after the specified incubation periods. Check at 7 days after incubation in same conditions.

- **Bibliography**

Kenner, G.A., Clark, H.F. and Kabler, P.W. (1961) J. Appl. Microbiol. 9: 15-20. Slanetz, L.W. and Bartley, C.H. (1957) J. Bact. 74: 591-595.

Chapman, G.H. (1947) J. Bact. 53: 504.

Nordic Committee on Food Analysis (1968) Leaflet 68.22/01/202022/01/202

- **Product use limitation**

This product is developed, designed and supplied exclusively for research use only. It is not intended for diagnostic applications or drug development, and it is not suitable for administration to humans or animals.