

Product: Coliform CV Selective Supplement

## Specification

A sterile selective supplement used for *E.coli/Coliform* isolation.

Presentation				
10 Freeze dried vials Vial with: 3 ± 0.1 g	<b>Packaging Details</b> 23x60 mm glass vials, tag labelled, White plastic cap - 10 vials per box.		Shelf Life 49 months	Storage 2-25 °C
Composition				
Compositon (g/vial)		NOTE : Each vial is sufficient to supplement 500 ml of medium Base:		
Cefsulodin Vancomycin		Chromogenic coliforms agar.		

### **Description /Technique**

Description:

The final purpose of this supplement is increase the selectivity of Chromogenic coliform Agar in order to detect total coliforms and E. coli in water and food samples.

Cefsulodin and Vancomycin suppress all the accompanying microbiota, especially Pseudomonas and Aeromonas spp.

<u>Technique:</u>

Collect, dilute and prepare samples and volumes as required according to specifications, directives, official standard regulations and/or expected results.

Reconstitute the vial with the sterile diluent in aseptic conditions and add it to 500 ml of melted Agar base cooled to 50°C. Do not overheat once suplemented.

Pour the complete medium into Petri dishes and, once solidified on a flat surface, spread the plates either by streaking or by spiral method.

Incubate the plates in aerobic atmosphere at 35 ± 2°C for 24-48h.

ncubation at 44±0.5 °C increases the selectivity of the medium and the specificity for E.coli isolation)

After incubation, count all the colonies that have appeared onto the surface of the agar, observing colour development when using a chromogenic base:

E. coli produces a dark blue to violet colonies due to the possession of two enzymes that cleaves the chromogenic substances.

Coliforms bacteria have only one enzyme so they can cleave only a substrate producing salmon/red colonies.

Total coliforms are the sum of E. coli colonies plus the salmon-red colonies.

Presumptive isolation of E.coli / Coliforms must be confirmed by further microbiological and biochemical tests.



Product: Coliform CV Selective Supplement

# **Quality control**

**Physical/Chemical control** Color : White-Gray

# Microbiological control

Reconstitute 1 vial as indicated in COMPOSITION; shake and dissolve completely

Add 1 vial to 500 ml of medium base. DO NOT HEAT once supplemented.

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Distribute the complete medium, cooled to 50 °C, into 90 mm plates

Incubate according instructions for complete medium indicated in COMPOSITION.

Aerobiosis. Incubation at 35 ± 2 °C, reading at 24-48 hours.

Microorganism	Growth
Escherichia coli ATCC® 25922, WDCM 00013	Good
Salmonella typhimurium ATCC <sup>®</sup> 14028, WDCM 00031	Good
Enterococcus faecalis ATCC <sup>®</sup> 29212, WDCM 00087	Inhibited
Stph. aureus ATCC <sup>®</sup> 25923, WDCM 00034	Inhibited
Ps. aeruginosa ATCC <sup>®</sup> 27853, WDCM 00025	Inhibited

#### Sterility control

100 ml TSB and 100 ml Thioglycollate. Incubation 48 h at 30-35 °C and 48 h at 20-25 °C: NO GROWTH. Check at 7 days after incubation in same conditions.

## Bibliography

· ADAMS, M., R.GRUBB, S.M. HAMER & A. CLIFFORD (1990) Colorimetric enumeration of Escherichia coli based on ß-glucuronidase activity. Appl. Environ. Microbiol. 56:2021.

· ISO 7704 Standard (1985) Water Quality - Evaluation of membrane filters used for microphiological analyses.

· KILIAN, M. & P. BÜLOW (1976) Rapid Diagnostic of Enterobacteriaceae. I. Detection of bacterial glycosidases. Acta Pathol. Microbiol. Scand. Sect. B 84:245-251.

· MANAFI, M & W. KNEIFEL (1989) A combined chromogenic-fluorogenic medium for the simultaneous detection of total coliform and E. coli in water. Zentralbl. Hyg. 189:225-234.

· MINISTERIO DE SANIDAD Y CONSUMO (2009) Orden SCO/778/2009 de 17 de marzo sobre métodos alternativos para el análisis microbiológico del agua de consumo humano. BOE. n.º 78 de 31-04-2009. Sección I, Págs. 30417-30420. Madrid.