

### Specification

Sterile selective supplement used for *Salmonella* isolation, according to ISO.

### Presentation

	Packaging Details	Shelf Life	Storage
10 Freeze dried vials Vial with: 3 ± 0.1 g	23x60 mm glass vials, tag labelled, White plastic cap - 10 vials per box.	49 months	2-25 °C

### Composition

Compositon (g/vial)

Novobiocin.....	0.0200
Brilliant Green.....	0.0050

NOTE : Each vial is sufficient to supplement  
500ml of Muller Kauffmann medium Base.

Reconstitute the original freeze-dried vial  
by adding:

Ethanol / Distilled water (3:3)..... 6 ml

### Description /Technique

#### Description:

Novobiocin+Brilliant green selective supplement is added to Muller-Kauffmann Tetratationate medium base in order to obtain a complete medium for the enrichment of enteric or intestinal pathogens, and for all the members of *Salmonella* type. Usually this medium is used for the analisis of polluted samples, like faeces, urine, waste water and others.

MKTTn was developed by Muller and later modified by Kauffmann with the addition of ox bile and brilliant green to improve selectivity. The addition of novobiocin was later described by Jeffries to improve inhibition of Proteus species.

#### Technique:

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

Reconstitute the vial with 6ml sterile diluent in aseptic conditions and add it to 500 ml of Muller Kauffmann Medium Base cooled to 50°C, previously added with Iodine solution.....4 g / l and Potassium iodide solution.....5 g / l.  
Do not overheat once supplemented.

Pour the complete medium into tubes and inoculate it.  
Incubate the tubes in aerobic atmosphere at 35 ± 2°C, lectura a las 18-24 horas.

Incubation times longer than those mentioned above or different incubation temperatures may be required depending on the sample or the specifications.

After incubation, observe turbidity appearing in the tubes.  
Subculture any confirmatory, secondary medium by streaking methodology or by spiral method, like , BGA, XLD, Hektoen...for *Salmonella* isolation.

Enumerate all the colonies that have appeared onto the surface of the agar.  
Presumptive isolation of *Salmonella sp.* must be confirmed by further microbiological and biochemical tests.

## Quality control

### Physical/Chemical control

Color : Green

### 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 10 ml tubes

Aerobiosis. Incubation at 37 ± 1 °C, reading after 24 ± 3 h

### Microorganism

*Enterococcus faecalis* ATCC® 29212, WDCM 00087

*Escherichia coli* ATCC® 8739, WDCM 00012

*S. typhimurium* (14028) + *E. coli* (8739) + *Ps.* (27853)

*S. enteritidis* (13076) + *E. coli* (8739) + *Ps.* (27853)

### Sterility control

Add 5 ml of the sample to:

100 ml TSB and 100 ml Thioglycollate.

Incubation 48 h at 30-35 °C and 48 h at 20-25 °C: NO GROWTH.

### Growth

Inhibition. Confirm in TSA at 37°C±1 reading 24 ± 3h.

Partially Inhibited ; ≤ 100 CFU Recovery in TSA

*Salmonella* coln. charact. in XLD (37°C±1 / 24 ± 3h) ≥ 10

*Salmonella* coln. charact. in XLD (37°C±1 / 24 ± 3h) ≥ 10

## Bibliography

- DIN Standard 10160 Untersuchung von Fleisch und Fleischerzeugnissen: Nachweis von Salmonellen. Referenzverfahren.
- DIN Standard 10181 Mikrobiologische Milchuntersuchung: Nachweis von Salmonellen. Referenzverfahren.
- DOWNES, F.P. & K.ITO (2001) Compendium of methods for the microbiological examination of foods. 4th ed. APHA. Washington. DC. USA.
- FDA (Food and Drug Administration) (1998) Bacteriological Analytical Manual. 8th ed. Revision A. AOAC International. Gaithersburg. MD. USA.
- FIL-IDF Standard 93 (2001) Milk and milk products: Research of Salmonella.
- HORWITZ, W. (2000) Official Methods of Analysis. 17th ed. AOAC International. Gaithersburg. MD. USA.
- ISENBERG, H.D. (1992) Clinical Microbiology Procedures Handbook. Vol. 1. APHA. Washington. DC. USA.
- ISO Standard 6579-1 (2017) Microbiology of food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1 : Detection of *Salmonella* spp.
- ISO Standard 6785 (2001) Milk and Milk Products - Detection of *Salmonella* spp.
- ISO Standard 3565 (1975) Meat Products: Reference Method for detection of *Salmonellae*.
- KAUFFMAN, F. (1931) Ein Kombiniertes Anreicherungsverfahren für Typhus und Paratyphus Bazillen. Zblt. Bakt Microbiol. Hyg Abt. I. Orig. 119:148.
- MARSHALL, R.T. (1993) Standard methods for the examination of dairy products. 16th ed. APHA Washington. DC. USA.
- MULLER, L. (1923) Un nouveau milieu d'enrichissement pour la recherche du bacille typhique est des paratyphiques. Comp. Rend. Soc. Biol. 89:434-437.
- U.S. PHARMACOPOEIA (2002) 25th ed. <61> Microbial Limits Test. US Pharmacopeial Convention Inc. Rockville. MD. USA.