

**Product :  
MULLER-KAUFFMANN TETRATHIONATE  
BROTH BASE**
**Also known as**

MKTTn

**Specification**

Medium used for the selective enrichment of salmonellae, according to ISO standards.

**Formula \* in g/L**

Bile salts No. 3.....	4.78	
Meat extract.....	4.30	(anhy.)..... 30.50 <sup>(*)</sup>
Casein peptone.....	8.60	
Sodium chloride.....	2.60	Final pH 8,0 ±0,2 at 25 °C
Calcium carbonate.....	38.70	
Sodium thiosulfate		(*1) equivalent to 47,80 g/L Sodium thiosulfate 5 H <sub>2</sub> O

\* Adjusted and /or supplemented as required to meet performance criteria

**Directions**

Add 89,48 g of powder to 1 L of distilled water. Bring to the boil and let it cool to 40-45°C. Add 20 mL of iodine/iodide solution and 2 vials of the Brilliant Green-Novobiocin (Art.DSHB3093) selective supplement and distribute into sterile tubes.

Do not reheat. The complete medium must be used immediately; the base, without iodine or antibiotic, may be stored in the refrigerator until needed.

White precipitate is due to calcium carbonate and does not effect the broths performance.

**Description**

 Tetrathionate Broth is a classic medium for the enrichment of enteric or intestinal pathogens, including all members of *Salmonella spp.*, from heavily polluted samples, such as faeces, urine, waste water and others. During preparation, when iodine is added, tetrathionate is produced from the sulfate, and this salt together with the bile salts in the medium, results in a strong inhibition of most of the normal intestinal bacteria, except for those which are capable of reducing tetrathionate, e.g. salmonellae. Reduction reactions liberate sulphuric acid, which is neutralized by the carbonate, avoiding a decrease in the pH, which is harmful even for salmonellae.

 However, many *Proteus* species resist the bile salt concentration and, they may reduce tetrathionate. So, many authors recommend the addition of other inhibitors simultaneously, such as 0,1% Brilliant Green Solution (10 mL/L) and/or novobiocin at 40 mg/L.

Medium Base can be kept indefinitely in the refrigerator, but after the addition of inhibitors, efficacy of the medium decreases with time.

With refrigeration the MKTTn with brilliant green, novobiocin added remains effective for 2 months but only 48 hours at 37°C. Once the iodine solution is added it only remains effective for 40 hours.

**Necessary supplement**

Brilliant Green + Novobiocin Selective Supplement (Art. No. DSHB3093)

Vial contents:

Necessary amount for 500 mL of complete medium.

Brilliant green 5,00 mg

Novobiocin, sodium salt 20,00 mg

Ethanol-Distilled water (1:20 Solvent)

**Technique**

The usual technique consists of adding the sample to the medium (1:10) and then homogenizing it well. Incubate à 37 ±1°C for 24h±3h after this time the medium loses its selectivity and the suppressed flora may also grow.

Some authors suggest incubation à 43°C and observations after 18, 24 and 48 hours, but one can get better results if a sample is taken from the surface of the broth after 30-36 hours.

Take aliquots with a loop and inoculate onto the surface of a selective media such as XLD Agar, SS Agar or Hektoen Enteric Agar, etc.

### Quality control

**Incubation temperature:** 37°C ±1,0

**Incubation time:** 24h±3h

**Inoculum:** Practical range 100 ± 20 CFU. Min. 50 CFU (Productivity) / 10<sup>4</sup>-10<sup>6</sup> CFU (Selectivity) according to ISO 11133:2014/Amd 1:2018

Microorganism	Growth	Remarks
<i>Enterococcus faecalis</i> ATCC® 29212	Inhibited	< 10 CFU Recovery in TSA
<i>Escherichia coli</i> ATCC® 8739	Partial Inhibition	≤ 100 CFU Recovery in TSA
<i>Salmonella typhimurium</i> ATCC® 14028+25922+27853		Good Recovery in XLD (Mixed cultures)
<i>Salmonella enteritidis</i> ATCC® 13076+25922+27853		Good Recovery in XLD (Mixed cultures)

### References

- 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.I.TO (2001) Compendium of methods for the microbiological examination of foods. 4th ed. APHA. Washington. DC. USA.
- FDA (Food and Drug Administrations) (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.
- ISO 11133:2014/ Adm 1:2018./ Adm1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- 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.

### Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).