

## MULLER KAUFFMANN TETRATHIONATE BROTH BASE ISO FORMULATION

### NOVOBIOCIN MKTT ANTIMICROBIC SUPPLEMENT

Powdered broth base and selective supplement  
for the enrichment of *Salmonella* spp. in foodstuffs

#### MUELLER KAUFFMANN TETRATHIONATE BROTH BASE ISO FORMULATION

##### TYPICAL FORMULA (g/L)

Enzymatic Digest of Meat	4.30
Enzymatic Digest of Casein	8.60
Sodium Chloride	2.60
Calcium Carbonate	38.70
Sodium Thiosulphate Anhydrous	30.30*
Ox Bile	4.78
Brilliant Green	9.60 mg

\*equivalent to 47.8 g of Sodium Thiosulphate Pentahydrate

#### NOVOBIOCIN MKTT ANTIMICROBIC SUPPLEMENT

##### VIAL CONTENTS (for 500 mL of medium)

Novobiocin.....20 mg

##### DIRECTIONS

Suspend 44,6 g in 500 mL of cold distilled water. Dissolve the ingredients by boiling the medium. Cool to 48-50° and add the contents of 1 vial of Novobiocin MKTT Antimicrobial Supplement (ref. n°4240047) and 10 mL of Iodine Solution (ref. n° 421501) Mix well and distribute 10 mL into sterile tubes.

Final pH at 25°C: 8.0 ± 0.2

If the Iodine Solution will be fresh prepared, proceed as follow:

Iodine solution:

Iodine..... 20g

Potassium Iodide ..... 25g

Distilled Water.....100 mL

Completely dissolve the potassium iodide in 10 mL of distilled water then add iodine and complete to 100 mL with sterile water. Add 10 mL to 500 mL of pre-cooled medium base.

##### DESCRIPTION

Muller Kauffmann Tetrathionate Broth Base ISO Formulation, supplemented with Iodine Solution and novobiocin 4mg/l (MKTTn) is recommended by ISO 6579 in the horizontal method for the selective enrichment of *Salmonella* spp. in food and animal feeding stuffs. The medium is to be used together with Rappaport Vassiliadis Soy (RVS) Broth and these selective enrichments are preceded by a non-selective enrichment in Buffered Peptone Water.

##### TECHNIQUE

The procedure recommended by ISO 6579:2002, is as follow:

Add 25g sample portion to 225mL of Buffered Peptone Water. If the required test portion is other than 25g, use a suitable quantity of Buffered Peptone Water to yield approximately 1/10 dilution (m/v).

Incubate the initial suspension at 37°C for a minimum of 16 hours and not more than 20 hours.

Transfer 0.1mL of the pre-enriched culture to a tube containing 10mL of Rappaport Vassiliadis Soy (RVS) Broth and 1mL to a flask containing 10mL of MKTTn.

Incubate the inoculated RVS Broth at 41.5°C +/- 1°C for 24hrs ± 3hrs.

Incubate the inoculated MKTTn at 37°C ± 1 for 24hrs ± 3.

Using a culture obtained from the RVS Broth inoculate by means of a 3mm loop; a large-size Petri dish or two 90mm Petri dishes containing XLD Medium (ref.n° 402206), proceed in the same way from the enrichment tube by inoculating a second plating medium (e.g. Chromogenic Salmonella Agar -ref. n° 405350), or another suitable selective *Salmonella* plating-out medium chosen by the laboratory).

Using the cultures obtained in MKTTn after 24 hours of incubation, repeat the procedure with the same two selective plating-out media.

Invert the dishes and incubate at 37°C for 24hrs. ± 3 hrs.

Examine for the presence of typical colonies. For confirmation take from each dish of each selective medium at least one typical or suspected colony and a further 4 colonies if the first is negative. Streak the selected colonies onto the surface of Nutrient Agar and incubate at 37°C for 24hrs. Use pure cultures for biochemical and serological confirmation. Biochemical confirmation tests include: TSI Agar, Urea Agar, L-Lysine Decarboxylase Medium, detection of  $\beta$ -galactosidase, VP reaction, indole detection. Serological confirmation includes the detection of the presence of *Salmonella* O-, Vi- and H antigens by slide agglutination test. Biochemical confirmation can be substituted with the rapid test MUCAP (code 191500). All the colonies MUCAP positive must be serologically confirmed.

**User quality assurance \*** (37°C - 24hrs, subculture on TSA or XLD)

Productivity Control

*S.typhimurium* ATCC 14028 or *S.enteritidis* ATCC 13076+ *E.coli* ATCC 25922 + *P.aeruginosa* ATCC 27853 : growth of *Salmonella* colonies after subculture on XLD Agar

Selectivity control

*E.coli* ATCC 25922 or ATCC 8739: completely inhibited after subculture on TSA*E.faecalis* ATCC 19433 or 29212 : completely inhibited after subculture on TSA

\* acc. to ISO/TS 11133-2

**STORAGE**

Dehydrated medium: 10°C / 30°C

User prepared medium base in flasks (without novobiocin and iodine solution): 1/ 5°C for 3 weeks

User prepared tubes: use within the day of preparation

Selective supplement: 2°C / 8°C

**REFERENCES**

- ISO 6579:2002 – Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp.
- ISO 6579:2002/Cor.1:2004
- ISO/TS 11133-2 Microbiology of food and animal feeding stuffs-Guidelines on preparation and production of culture media-Part 2 Practical guidelines on performances testing culture media

**PACKAGING****4017452**      **M.K. Tetrathionate Broth Base ISO Form.**      **500 g ( 5.6 L)****4017454**      **M.K. Tetrathionate Broth Base ISO Form.**      **5 kg (56 L)****4240047**      **Novobiocin MKTT Antimicrobial Suppl. (20 mg)** 10 vials, each for 500 mL of medium