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**MULLER KAUFFMANN TETRATHIONATE BROTH BASE  
ISO FORMULATION  
NOVOBIOCIN MKTT ANTIMICROBIC SUPPLEMENT  
IODINE SOLUTION  
MK TETRATHIONATE NOVOBIOCIN BROTH  
Dehydrated culture medium, selective supplements and ready to use tubes**

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**1 - INTENDED USE**

Selective liquid medium and supplements for the enrichment of *Salmonella* from food and animal feeding stuffs.

**2 - COMPOSITION - TYPICAL FORMULA\*  
(AFTER RECONSTITUTION WITH 1 L OF WATER)****DEHYDRATED MEDIUM**

Enzymatic digest of meat	4.30 g
Enzymatic digest of casein	8.60 g
Sodium chloride	2.60 g
Calcium carbonate	38.70 g
Sodium thiosulphate anhydrous	30.30 g ^
Ox bile	4.78 g
Brilliant green	9.60 mg

\*Equivalent to 47.8 g of sodium thiosulphate pentahydrate

\*The formula may be adjusted and/or supplemented to meet the required performances criteria.

**NOVOBIOCIN MKTT SELECTIVE SUPPLEMENT****VIAL CONTENT (FOR 500 mL OF MEDIUM)**

Novobiocin	20 mg
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**IODINE SOLUTION****FLASK CONTENT (50 mL)**

Iodine	10.0 g
Potassium iodide	12.5 g
Purified water	50 mL

**MK TETRATHIONATE NOVOBIOCIN BROTH****READY-TO-USE TUBES**

Muller Kauffmann Tetrathionate Broth Base	1000 mL
Novobiocin	40 mg
Iodine Solution	10 mL

**3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE**

Muller Kauffmann Tetrathionate (MKTT) Broth was originally described by Muller<sup>1</sup> for the isolation of salmonellae and later modified by Kauffmann<sup>2</sup> by the inclusion of ox bile and brilliant green as selective agents to suppress bacteria such as *Proteus* spp. Jeffries<sup>3</sup> proposed the addition of novobiocin at 40 mg per litre of broth to improve the selective properties of the medium.

The detection of *Salmonella* in foods necessitates four successive stages: pre-enrichment in non-selective liquid medium, enrichment in two selective liquid media, plating out and recognition, confirmation.

MKTT Broth Base with added novobiocin and iodine solution is used for the selective enrichment of *Salmonella* from food chain samples, together with RVS Broth or MSRV Agar, and meets the requirements of ISO 6579.<sup>4</sup>

Peptones provide carbon, nitrogen, vitamins and minerals for microbial growth; the selective agents of the medium are ox bile, brilliant green and sodium tetrathionate which is formed from sodium thiosulfate when the iodine/potassium iodide solution is added to the medium; calcium carbonate neutralizes the sulfuric acid that is produced by the reduction of tetrathionate during the growth of salmonellae, keeping the pH at neutral values. Novobiocin is active mostly against Gram-positive bacteria but also against a few Gram-negative bacteria. The complete medium allows the development of salmonellae and it is inhibitory for Gram-positive bacteria and for a large part of Gram-negative bacteria of enteric origin.

**4 - DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION**

Suspend 44.6 g in 500 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Avoid overheating, do not autoclave. Cool to 47-50° and add the contents of 1 vial of Novobiocin MKTT Antimicrobial Supplement (ref. n°4240047) reconstituted with 5 mL of sterile purified water and 10 mL of Iodine Solution (ref. n° 421501). Mix well and aseptically distribute 10 mL into sterile tubes.

**5 - PHYSICAL CHARACTERISTICS**

Dehydrated medium appearance	pale green, fine, homogeneous, free-flowing powder.
Solution and prepared tubes appearance	pale green with white precipitate.
Freeze-dried novobiocin supplement appearance	short, dense white pellet; after reconstitution colourless limpid solution.
Liquid iodine solution appearance	brown, limpid.
Final pH of the complete medium at 20-25°C	8.0 ± 0.2



**6 - MATERIALS PROVIDED - PACKAGING**

Product	Type	REF	Pack
Muller Kauffmann Tetrathionate Broth Base	Dehydrated culture medium	4017452 4017454	500 g (5.6 L) 5 kg (56 L)
Novobiocin MKTT Selective Supplement	Freeze-dried supplement	4240047	10 vials, each for 500 mL of medium
Iodine Solution	Liquid supplement	421501	50 mL
MK Tetrathionate Novobiocin Broth	Ready-to-use tubes	551745	20 x 10 mL

**7 - MATERIALS REQUIRED BUT NOT PROVIDED**

Water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile tubes, ancillary culture media and reagents.

**8 - SPECIMENS**

Food, feed, food chain samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.<sup>4</sup>

**9 - TEST PROCEDURE**

The following method is a summary taken from the ISO 6579.<sup>4</sup>

1. Prepare the test sample in accordance with the specific International Standard dealing with the product concerned. In general, an amount of test portion is added to a quantity of pre-warmed Buffered Peptone Water (REF 401278) to yield a tenfold dilution (e.g., 25 g test portion is mixed with 225 mL of Buffered Peptone Water).
2. Incubate between 34 °C and 38 °C for 18 h ± 2 h.
3. Transfer 0.1 mL of the culture obtained in Buffered Peptone Water to a tube containing 10 mL of the RVS broth (REF 401981) or to the surface of a MSRV Agar plate (REF 401982).
4. Transfer 1 mL of the culture obtained in Buffered Peptone Water to a tube containing 10 mL of Muller Kauffmann Tetrathionate Novobiocin Broth (REF 401745 - MKTTn Broth).
5. Incubate the inoculated RVS Broth (or MSRV plates) at 41.5 °C ± 1 for 24 h ± 3 h.
6. Incubate the inoculated MKTTn Broth between 34 °C and 38 °C for 24 h ± 3 h.
7. From RVS Broth or MSRV medium and MKTTn Broth transfer a loopful of growth on a plate of XLD Agar ISO Formulation and on another selective medium for *Salmonella* based on different diagnostic characteristics to those of XLD agar (e.g. Chromogenic Salmonella Agar REF 405350). With MSRV medium positive plates use a 1 µL loop, with MKTTn Broth use a 10 µL loop.
8. Incubate the XLD Agar ISO Formulation inverted between 34 °C and 38 °C and examined after 24 h. Incubate the second selective plating-out medium in accordance with the instructions for use.

**NOTES**

After incubation, it is permissible to store the pre-enriched sample and selective enrichment at 2-8 °C for a maximum of 72 h.<sup>4</sup>

In dried milk products and cheese, *Salmonella* may be sub lethally injured. Incubate the selective enrichment media from these products for an additional 24 h ± 3 h. When investigating outbreak samples, this additional incubation time may also be beneficial.<sup>4</sup>

**10 - READING AND INTERPRETATION**

After incubation, growth of organisms is indicated by turbidity and discolouration.

Refer to the instructions for use of the two plated media for the description of *Salmonella* colony characteristics.

Mark suspect colonies on each plate. Select suspect colonies for subculture and confirmation.

Biochemical confirmation tests include: TSI Agar, Urea Agar, L-Lysine Decarboxylase Medium, detection of β-galactosidase (optional), indole detection (optional).<sup>4</sup> Serological confirmation includes the detection of the presence of *Salmonella* O- and H-antigens.

Biochemical confirmation can be substituted with the rapid MUCAP Test (REF 191500). All the colonies MUCAP Test positive must be serologically confirmed.

**11 - USER QUALITY CONTROL**

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control of medium.

CONTROL STRAINS	INCUBATION T° / T / ATM	EXPECTED RESULTS
S. Typhimurium ATCC 14028 + P. aeruginosa ATCC 27853 + E. coli ATCC 25922	34-38°C / 24 h ± 3 h A	> 10 typical colonies after subculture on XLD Agar
E. faecalis ATCC 29212 E. coli ATCC 25922	34-38°C / 24 h ± 3 h A	< 100 colonies after subculture on TSA partially inhibited after subculture on TSA

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

**12 - PERFORMANCES CHARACTERISTICS**

Prior to release for sale a representative sample of all lots of MKTTn ready to use tube and dehydrated MKTT Broth Base, supplemented with novobiocin and iodine solution, are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.

Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of target organisms in test tubes, incubating at 35-37°C for 24 hours, sub-culturing on Tryptic Soy Agar plates and recording the highest dilution showing growth in Reference Batch (G<sub>RB</sub>) and in Test Batch (G<sub>TB</sub>). Productivity is tested with the following target strains: S. Typhimurium ATCC 14208, S. Enteritidis ATCC 13076, The productivity index G<sub>RB</sub>-G<sub>TB</sub> for each test strain shall be ≤ 1.

Productivity and selectivity are tested together with mixtures of approximately 100 CFU of target organisms and 1000 CFU of non- target organisms per test tubes, incubating 35-37°C for 24 hours. Mixtures of target and non-target strains: S. Typhimurium ATCC 14028 +E. coli





ATCC 25922+*P. aeruginosa* ATCC 27853. After incubation of inoculated tubes and sub-culture on XLD Agar plates, the target strains will show more than 10 colonies per plate.

Moreover, selectivity is evaluated by inoculating approximately 10,000 CFU/tube of non-target organisms and incubating at 35-37°C for 24 hours and sub-culturing on Tryptic Soy Agar plates. Selectivity is tested with the following non-target strains: *E. coli* ATCC 25922, *E. faecalis* ATCC 2921. CFUs of *E. coli* shall be less than 100 while CFUs of *E. faecalis* shall be less than 10 on the sub-cultured plates of Tryptic Soy Agar.

### 13 - LIMITATIONS OF THE METHOD

- Muller Kauffmann Tetrathionate Broth is not suitable for growth of *S. Typhi*, *S. Paratyphi*, *S. Sendai*, *S. Gallinarum*; <sup>5</sup>
- Colonies of presumptive *Salmonella* must be sub cultured and their identity confirmed by means of appropriate biochemical and serological tests.

### 14 - PRECAUTIONS AND WARNINGS

- The medium base, the supplement and the ready-to-use tubes are for microbiological control and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplements shall be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated medium and supplements are classified as hazardous and must be handled with suitable protection. Before use, consult the Material Safety Data Sheets.
- This culture medium contains raw materials of animal origin. The ante and post mortem controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website [www.biolifeitaliana.it](http://www.biolifeitaliana.it), describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Be careful when opening screw cap tubes to prevent injury due to breakage of glass. Be careful when opening the metal ring of supplement vial to avoid injury.
- The novobiocin supplement is sterilized by membrane filtration.
- Each tube of this culture medium is for single use only.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplements as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheets are available on the website [www.biolifeitaliana.it](http://www.biolifeitaliana.it).
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

### 15 - STORAGE CONDITIONS AND SHELF LIFE

#### Ready-to-use medium in tubes

Upon receipt, store tubes in their original pack at 2-8°C away from direct light. If properly stored, the tubes may be used up to the expiration date. Do not use the tubes beyond this date. Tubes from opened secondary packages can be used up to the expiration date. Opened tubes must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use tubes with signs of deterioration (e.g., microbial contamination, abnormal turbidity, precipitate, atypical colour).

#### Dehydrated medium

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

#### Freeze-dried novobiocin supplement

Upon receipt, store the product in the original package at 2-8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilized product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

#### Liquid iodine supplement

Upon receipt, store the product in the original package at 2-8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Before use, examine the solution and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (tubes/bottles) and the applied storage conditions (temperature and packaging). According to ISO 6579 the base medium may be stored in closed flasks at 2-8 °C for up to three months. If the complete medium is not used immediately, store it in the dark at 2-8°C. The pH may drop during storage due to chemical reactions. Do not use the complete medium if the pH drops below 7.0.<sup>4</sup>

### 16 – REFERENCES

1. Muller L. A nouveau milieu d'enrichissement pour la recherche du bacille typhique e des paratyphiques. C.R. Soc. Biol. (Paris) 1923; 89:434-443
2. Kauffmann F. Weitere Erfahrungen mit den kombinierten Anreicherungsverfahren für Salmonellabacillen. Z Hyg Infektionskr. 1935; 117: 26-32
3. Jeffries L. Novobiocin-tetrathionate broth: a medium of improved selectivity for the isolation of Salmonellae from faeces. J Clin Pathol 1959; Nov;12(6):568-71.
4. ISO 6579-1:2017-2. Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella —Part 1: Detection of Salmonella spp.





5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

### TABLE OF APPLICABLE SYMBOLS

<b>REF</b> or <b>REF</b> Catalogue number	<b>LOT</b> Batch code	 Manufacturer	 This side up	 Store in a dry place	 Fragile
 Temperature limitation	 Content sufficient for <n> tests	 Consult Instructions for Use	 Use by	 Keep away from direct light	 For single use only

### REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2022/08

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

