MULLER-KAUFFMANN BROTH BASE WITH BRILLIANT GREEN AND NOVOBIOCIN (MKTTN)

MKFB-00I-500

- **Principle**

Recommended by the ISO 6579 and ISO 19250 norms to be used as a selective enrichment broth for the detection of *Salmonella* spp in all food types, including milk and dairy products, molluscan shellfish and other fish products, and in water samples and environmental swabs.

Beef extract and casein peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Calcium carbonate is a neutralizer which absorbs toxic metabolites. Bile salt, brilliant green and novobiocin inhibit organisms other than Salmonella. Selectivity is also obtained by both sodium thiosulfate and tetrathionate, suppressing coliforms. Tetrathionate is formed in the medium with the addition of the iodine and potassium iodide solution. Organisms containing the enzyme tetrathionate reductase will thrive in this medium. Sodium chloride supplies essential electrolytes for transport and osmotic balance.

- **Regulatory compliance**

This product is manufactured under a quality management system in accordance with ISO 9001 and ISO 13485, and its formulation and quality control comply with applicable international standards, such as ISO 11133, where relevant.

For this specific medium, compliance is also established with the relevant requirements of ISO 19250 and ISO 6579.

- **Composition**

Ingredients	**g/L**
Enzymatic digest of casein	8.60
Brilliant green	0.0096
Novobiocin	0.04
Sodium chloride	2.60
Beef extract	4.30
Calcium carbonate	38.70
Ox Bile	4.78
Sodium thiosulfate	30.50

- **Preparation**

Suspend 89.53 grams of the medium in one litre of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE. Cool to 45-50 °C.

Aseptically Add 20 ml of an iodine and potassium iodide solution (20 g of iodine and 25 g of potassium iodide in 100 ml of sterile distilled water). Homogenize gently and dispense into sterile containers.

- **Applications and use**

* For detection of *Salmonella* spp in food, animal feed, animal faeces, and environmental samples according to ISO 6579:

- Preenrichment in non-selective liquid medium: Inoculate the Buffered Peptone Water (AGPT-0DI-500) with the sample or dilutions and incubate at 34-38 °C for 18±2 h.

- Enrichment in/on selective media: Inoculate, with the culture obtained in the pre-enrichment stage, the Rappaport Soy Broth (Vassiliadis)(RSVB-00I-500) and the MKKTN Broth. The Rappaport Soy Broth is incubated at 41.5 °C for 24±3 h, and the MKKTN Broth at 34-38 °C for 24±3 h.

- Plating out on selective solid media: From the selective enriched cultures, inoculate two selective isolation agars; XLD agar (AGXL-00I-500) and any other selective medium complementary to XLD agar, such as Brilliant Green Agar (BGAG-00I-500), Hektoen Enteric Agar (HEAG-00P-500) and Salmonella Shigella Agar (SSAG-IEP-500). Incubate the XLD plates inverted at 34-38 °C for 24±3 h. Incubate the second selective medium in accordance with the manufacturer's instructions.

- Confirmation: Subculture colonies of presumptive Salmonella and confirm their identity by biochemicals and serological tests.

Note: According to Annex D of ISO 6579-1: 2017, for the detection of enterica subspecies enterica serovars Typhi and Paratiphy, Selenite Cystine Broth should be added as a selective enrichment medium, and Bismuth Sulphite Agar (Wilson Blair) should be selected as a second selective medium.

* For detection of *Salmonella* spp in water samples according to ISO 19250:

- Preenrichment in non-selective medium: Inoculate the Buffered Peptone Water (AGPT-0DI-500) with the sample or dilutions and incubate at 34-38 °C for 18±2 h.

- Enrichment in selective media: Inoculate, with the culture obtained in the pre-enrichment stage, the Rappaport Soy Broth (Vassiliadis)(RSVB-00I-500) and the MKKTN Broth. The Rappaport Soy Broth is incubated at 41.5±1 °C and the MKKTN Broth at 34-38 °C, both for 24±3 hours.

- Plating out on selective solid media: From the selective enriched cultures, inoculate two selective isolation agars; XLD agar (AGXL-00I-500) and any other selective medium complementary to XLD agar (For instance, Brilliant Green Agar (BGAG-00I-500)). Incubate the XLD plates inverted at 34-38 °C for 24±3 hours. Incubate the second selective medium in accordance with the manufacturer's instructions.

- Confirmation: Subculture colonies of presumptive Salmonella and confirm their identity by biochemicals and serological tests.

- **Quality control**

Solubility	w/o rests
Appearance	Fine powder
Colour of the dehydrated medium	White

Colour of the prepared medium	Green-blue
Final pH (25 °C)	8.0 ± 0.2

- **Microbiological test**

According to ISO 11133:

Incubation conditions: 34-38 °C /24±3 h.

Inoculation conditions: Target microorganisms (1000 CFU) / Selectivity (10^4-10^6 CFU).

Microorganisms	ATCC	Specification	Characteristic reaction
Salmonella typhimurium + *Escherichia coli* + *Pseudomonas aeruginosa*	14028 + 8739 + 27853	> 10 colonies on XLD or other medium of choice	Colonies with black centre and a light transparent zone of reddish colour due to the colour change of the medium
Salmonella enteritidis + *Escherichia coli* + *Pseudomonas aeruginosa*	13076 + 8739 + 27853	> 10 colonies on XLD or other medium of choice	Colonies with black centre and a light transparent zone of reddish colour due to the colour change of the medium
Enterococcus faecalis	29212	< 10 colonies on TSA	-
Escherichia coli	8739	Partial inhibition <100 colonies on TSA	-

- **Storage**

The product is highly hygroscopic; keep the container always closed and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Temp. Min.:2 °C Temp. Max.:25 °C.

Note: Sterilize media immediately after reconstitution.

- **Bibliography**

ISO 6579 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Salmonella spp

ISO 6579 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Salmonella spp. Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC

Kauffmann, F. 1935. Weitere erfahrungen mit dem kombininierten anreicherungsverfahren fur Salmonella bazillen. Ztschr. F. Hyg. 117: 26-32. ISO 19250 water quality-detection of Salmonella spp

- **Product use limitation**

This product is developed, designed and supplied exclusively for research use only. It is not intended for diagnostic applications or drug development, and it is not suitable for administration to humans or animals.