



## PRODUCT SPECIFICATION SHEET

### Mueller Kauffman Tetrathionate Novobiocin Broth Base (DM80950)

#### Intended Use

Mueller Kauffman Tetrathionate Novobiocin Broth Base (DM80950) is recommended for improved enrichment and isolation of *salmonellae* as per ISO 6579:2002.

#### Product Summary and Explanation

Salmonellosis is one of the most important and most frequently reported human foodborne diseases worldwide.<sup>(1)</sup> Outbreaks have been associated with the consumption of pork and pork products,<sup>(2, 3)</sup> broiler chickens,<sup>(4)</sup> and other animals. Environmental sources include animal feed, litter and dust from hen houses, and animal feces. The process of isolating *Salmonella* from food is often difficult. The examination of various types of food products for *Salmonella* requires methods different from those used in clinical laboratories. The need for such method is due to the generally low numbers of *Salmonellae* in foods, accompanied by larger numbers of other contaminating bacteria and the frequently poor physiological state of these pathogens following exposure to stressful conditions during food processing or storage. Pre-enrichment is necessary to permit the detection of low numbers of *Salmonella* or injured *Salmonella*. Injured *Salmonella* are resuscitated in non-selective broth medium, which facilitates detection of sublethally injured *Salmonella*. The ideal pre-enrichment broth should provide for the repair of cell damage, dilute toxic or inhibitory substances and nutritive enough to favour growth of *Salmonella*. When analysing *Salmonella* from food, pre-enrichment cultures are usually incubated at 35-37°C for 18-24 hours and then a portion is subcultured to one or more selective enrichment broths. Usually, 1 ml of pre-enrichment culture is inoculated to 9 ml of selective enrichment broth. Selective enrichment media comprises of selective ingredients that allow the proliferation of *Salmonella* and inhibit the growth of competing non-salmonella microorganisms. BAM recommends Lactose Broth (DM527) for pre-enrichment of *Salmonella* from food. Tetrathionate Broth and Rappaport Vassiliadis Medium are used for selective enrichment. Various modifications of Tetrathionate Broth have generally found wider applications for the detection of foodborne *Salmonella*.<sup>(5)</sup> Mueller<sup>(6)</sup> recommended Tetrathionate Broth as a selective medium for the isolation of *Salmonella*. Kauffman<sup>(7)</sup> modified the formula by addition of ox bile and brilliant green as selective agents to suppress bacteria such as *Proteus* species. Jeffries<sup>(8)</sup> described the addition of novobiocin at 40 mg per liter of broth to further suppress the growth of *Proteus* sp. The British Standard Specification specifies Brilliant Green Tetrathionate Broth for isolating *Salmonella* from meat and meat products and from poultry and poultry products.<sup>(9)</sup> It is also a recommended selective broth for isolating *Salmonella* from animal feces, environmental samples and sewage-polluted water.<sup>(10-14)</sup> Selectivity is conferred by tetrathionate (from the reaction of thiosulphate and iodine). Using more than one selective broth increases the isolation of *Salmonella* from samples with multiple serotypes.<sup>(15)</sup> Mueller Kauffman Tetrathionate Broth Base conforms to ISO specifications.<sup>(16)</sup>

#### Principles of the Procedure

Mueller Kauffman Tetrathionate Novobiocin Broth Base contains casein enzymic hydrolysate and peptic digest of animal tissue as sources of carbon, nitrogen, vitamins and minerals essential for growth. Ox bile and added brilliant green are selective agents, which inhibit gram-positive and other gram-negative organisms. Calcium carbonate is the buffering agent. Sodium chloride maintains osmotic equilibrium. Sodium thiosulphate is a source of sulfur. The tetrathionate (S<sub>4</sub>O<sub>6</sub>) anions constitute the principle selective agent in these enrichment media. If desired, 4 mg of novobiocin per litre of broth can be added to suppress *Proteus* species.<sup>(8)</sup>

#### Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	4.30
Casein enzymic hydrolysate	8.60
Ox bile	4.75
Sodium chloride	2.60
Calcium carbonate	38.70





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Sodium thiosulphate, pentahydrate	47.80
Brilliant green	0.0095
Final pH: 8.2 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

### Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. Due to presence of calcium carbonate, the prepared media forms opalescent solution with white precipitate

### Directions

1. Suspend 89.42 grams of dehydrated medium in one liter distilled water.
2. Heat the medium just to boiling.
3. DO NOT AUTOCLAVE.
4. Cool to 45-50°C and just before use aseptically add 20 ml of iodine solution (20 gram iodine and 25 gram potassium iodide in 100 ml sterile distilled water) along with rehydrated contents of 1 vial of MKTT Novobiocin Supplement (MS188).
5. Mix well to disperse calcium carbonate uniformly before dispensing in sterile tubes.

### Quality Control Specifications

Dehydrated Appearance	Cream to greenish yellow homogeneous free flowing powder
Prepared Medium	Light green coloured opalescent solution forms with heavy white precipitate
Reaction of 8.93% Solution	pH : 8.2 ± 0.2 at 25°C
Gel Strength	Not Applicable

**Expected Cultural Response:** Cultural characteristics observed after an incubation at 43°C for 18-48 hours with added 20ml iodine solution and MKTT Novobiocin Supplement (MS188), when subcultured on Soyabean Casein Digest Agar (M247).

Sr. No.	Organisms	Results to be achieved	
		Inoculum (CFU)	Recovery
1.	<i>Escherichia coli</i> ATCC 25922	50-100	none-poor
2.	<i>Proteus vulgaris</i> ATCC 13315	50-100	none-poor
3.	<i>Shigella flexneri</i> ATCC 12022	>=10 <sup>3</sup>	inhibited
4.	<i>Salmonella enteritidis</i> ATCC 13076	50-100	excellent
5.	<i>Salmonella enteritidis</i> ATCC 13076	50-100	excellent
6.	<i>Salmonella paratyphi A</i> ATCC 9150	50-100	excellent
7.	<i>Salmonella paratyphi B</i> ATCC 8759	50-100	excellent
8.	<i>Salmonella typhi</i> ATCC 6539	>=10 <sup>3</sup>	inhibited
9.	<i>Salmonella typhimurium</i> ATCC 14028	50-100	excellent

The organisms listed are the minimum that should be used for quality control testing.

### Test Procedure

1. Add approximately 10 grams of sample to 100 ml of broth.
2. Shake well and place the flask in a 45°C water bath for 15 minutes.
3. Remove the flasks and place in an incubator or water bath at 43°C.
4. Several studies have shown increased recovery of *Salmonella* following incubation of selective enrichment at 43°C.<sup>(17)</sup>





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5. After an incubation for 18-24 hours and 48 hours, subculture on Brilliant Green Agar, Modified (DM044). This medium is not suitable for the growth of *Salmonella typhi*, *Salmonella sendai*, and *Salmonella pullorum* etc.

### Results

1. *Salmonella* spp. will produce red colonies with good growth.
2. Refer appropriate references and procedures for interpretation of results.

### Storage

Store the sealed bottle containing the dehydrated medium at 10- 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

### Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

### Limitations of the Procedure

1. This medium is not suitable for the growth of *Salmonella typhi*, *Salmonella sendai*, and *Salmonella pullorum* etc.
2. The complete medium is unstable and should be used immediately.
3. It may be stored at 2-8°C in the dark for no more than 7 days.
4. Organisms other than *Salmonellae*, such as *Morganella morganii* and some *Enterobacteriaceae* may grow in the medium.
5. Therefore, confirmatory tests should be carried out on all presumptive *Salmonella* colonies that are recovered.
6. Consult appropriate texts for detailed information and recommended procedures.

### Packaging

**Product Name : Mueller Kauffman Tetrathionate Novobiocin Broth Base**

**Product Code : DM80950**

**Available Pack sizes : 500gm**

### References

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### Further Information

For further information please contact your local MICROMASTER Representative.



**MICROMASTER LABORATORIES PRIVATE LIMITED**

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Unit 38/39, Kalpataru Industrial Estate,  
Near Runwal Estate, Behind 'R-Mall', Ghodbunder Raod,  
Thane (W) - 400607. M.S. INDIA.

Ph: +91-22-25895505, 4760, Cell: 9320126789.

Email: [micromaster@micromasterlab.com](mailto:micromaster@micromasterlab.com)  
[sales@micromasterlab.com](mailto:sales@micromasterlab.com)

Prepared By	Checked By	Approved By
 01.01.2018	 01.01.2018	 01.01.2018
<b>Microbiologist</b>	<b>Head Quality Control</b>	<b>Head Quality Assurance</b>

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