

# Muller Kauffmann Tetrathionate Broth Base

## Intended Use

Muller Kauffmann Tetrathionate Broth Base is used for enriching *Salmonella* from food and environmental samples prior to selective isolation.

## 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 key to successful recovery lies in obtaining the contaminated portion of test sample. Even when the contaminated material has been obtained, *Salmonella* may be present only in small numbers and accompanied by larger numbers of other contaminating bacteria. Pre-enrichment is necessary to permit the detection of low numbers of *Salmonella* or injured *Salmonella*. Following pre-enrichment, the selective enrichment step destroys most of the competing flora, allowing the *Salmonella* to be recovered.

Muller<sup>5</sup> recommended Tetrathionate Broth as a selective medium for the isolation of *Salmonella*. Kauffmann<sup>6</sup> modified the formula to include oxbile and brilliant green as selective agents to suppress bacteria such as *Proteus* spp. Jeffries<sup>7</sup> described the addition of novobiocin at 40 mg per liter of broth to further suppress the growth of *Proteus* sp. Muller Kauffmann Tetrathionate Broth Base is used for isolating *Salmonella* from food, environmental samples<sup>4,8-13</sup> and animal feces.<sup>14</sup> Using more than one selective broth increases the isolation of *Salmonella* from samples with multiple serotypes.<sup>15</sup>

## Principles of the Procedure

Muller Kauffmann Tetrathionate Broth Base contains peptone and beef extract as sources of carbon, nitrogen, vitamins and minerals. Oxgall and added brilliant green are selective agents which inhibit gram-positive and other gram-negative organisms. Calcium carbonate is the buffering agent. Sodium thiosulfate is a source of sulfur.

## Formula

### Difco™ Muller Kauffmann Tetrathionate Broth Base

Approximate Formula* Per Liter	
Beef Extract.....	5.0 g
Peptone .....	10.0 g
Sodium Chloride .....	3.0 g
Calcium Carbonate .....	45.0 g
Sodium Thiosulfate (anhydrous) .....	38.1 g
Oxgall .....	4.7 g

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

Dehydrated Appearance:	Off-white to light beige, free-flowing, homogeneous.
Solution:	10.58% solution, insoluble in purified water. Solution is very pale green with white precipitate.
Prepared Appearance:	Very pale green with white precipitate.
Reaction of 10.58% Solution, with additives, at 25°C:	pH 7.0 ± 0.2 (adjusted)

## Cultural Response

### Difco™ Muller Kauffmann Tetrathionate Broth Base

Prepare the medium per label directions, with the addition of 1.9 mL iodine solution and 0.95 mL brilliant green solution per 100 mL of medium. Inoculate and incubate at 42-43°C for 18-24 hours. Subculture to Brilliant Green Agar and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
<i>Escherichia coli</i>	25922	10 <sup>3</sup> -2 × 10 <sup>3</sup>	None to poor	–
<i>Proteus vulgaris</i>	13315	10 <sup>3</sup> -2 × 10 <sup>3</sup>	None to poor	–
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	100-300	Good	Red
<i>Salmonella senftenberg</i> (NCTC 10384)		100-300	Good	Red

## Directions for Preparation from Dehydrated Product

1. Suspend 105.8 g of the powder in 1 L of purified water and boil gently.
2. Cool to below 45°C.
3. Add 19 mL of iodine solution (20 g iodine and 25 g potassium iodide in 100 mL water) and 9.5 mL brilliant green solution (0.1 g brilliant green in 100 mL water).
4. Adjust the pH of the complete medium to 7.0 ± 0.2 using 1N HCl.
5. Dispense into sterile tubes, mixing well to evenly disperse the calcium carbonate.
6. Test samples of the finished product for performance using stable, typical control cultures.

## Procedure

Refer to appropriate references for details on sample collection and preparation according to sample type and geographic location.<sup>4,8-13</sup>

Consult appropriate references for details on test methods using Muller Kauffmann Tetrathionate Broth.<sup>4,8-13</sup>

## Expected Results

*Salmonella* spp. will produce red colonies with good growth.

## Limitations of the Procedure

1. The complete medium is unstable and should be used immediately. It may be stored at 2-8°C in the dark for no more than 7 days.
2. Due to the nutritional requirements and inhibitory characteristics of the organisms themselves, organisms other than salmonellae, such as *Morganella morganii* and some *Enterobacteriaceae* may grow in the medium.
3. Confirmatory tests, such as fermentation and seroagglutination reactions, should be carried out on all presumptive *Salmonella* colonies that are recovered.

## References

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## Availability

### Difco™ Muller Kauffmann Tetrathionate Broth Base

Cat. No. 218531 Dehydrated – 500 g

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## User Quality Control

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### Identity Specifications

Difco™ Muller Kauffmann Tetrathionate Broth Base