

89176 Muller-Kauffmann Tetrathionate Novobiocin Broth

Mueller Kauffman Tetrathionate Novobiocin Broth is used for improved enrichment and isolation of *Salmonellae*.

Composition:

Ingredients	Grams/Litre
Peptic digest of animal tissue	4.3
Casein enzymic hydrolysate	8.6
Ox bile	4.75
Sodium chloride	2.6
Calcium carbonate	38.7
Sodium thiosulphate, pentahydrate	47.8
Brilliant green	0.0095
Final pH 8.2 +/- 0.2 at 25°C	

The prepared 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. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Directions:

Suspend 89.42 grams of dehydrated medium I in 1000 ml distilled water. Heat the medium just to boiling. DO NOT AUTOCLAVE. 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 (Cat. No 79894). Mix well to disperse calcium carbonate uniformly before dispensing in sterile tubes.

Note: Due to presence of calcium carbonate, the prepared media forms opalescent solution with white precipitate.

Principle and Interpretation:

Due to low numbers of *Salmonellae* in foods and the fact that many cells are injured during food processing it is important to rescue and enrich the bacterial cells with non-selective broth medium. Sublethally injured *Salmonella* can recover and proliferate in an ideal pre-enrichment broth. After the pre-enrichment step cultures are usually sub cultured to one or more selective enrichment broths. Normally 1 ml of pre-enrichment culture is inoculated to 9 ml of selective enrichment broths, which allow the growth of *Salmonella* and inhibit other non-salmonella microorganisms. Lactose Broth (94792) is recommended by BAM for pre-enrichment of *Salmonella* from food. Further a selective enrichment step is recommended in Tetrathionate Broth and Rappaport Vassiliadis Medium. Various modifications of Tetrathionate Broth exist for the detection of *Salmonella* (7). Mueller (1) recommended Tetrathionate Broth as a selective medium for the isolation of *Salmonella* and Kauffman (2) modified the formula by adding ox bile and brilliant green as selective agents to suppress other bacteria like e.g. *Proteus* species. The ISO recommends Brilliant Green Tetrathionate Broth for the isolation of *Salmonella* from meat, meat products, poultry and poultry products (3). It is also recommended as selective broth for isolation of *Salmonella* from animal feces and sewage-polluted water (4). Selectivity is conferred by tetrathionate (from the reaction of thiosulphate and iodine). Mueller Kauffman Tetrathionate Novobiocin Broth contains peptic digest of animal tissue and casein enzymic hydrolysate as sources of carbon, nitrogen, vitamins and minerals. Ox bile and brilliant green are selective agents, which inhibits gram-positive and most other gram-negative organisms.



Proteus species can be inhibited by addition of novobiocin (6). Sodium chloride maintains the osmotic balance while calcium carbonate act as the buffering agent. Sodium thiosulphate is a source of sulfur and the tetrathionate (S₄O₆) anions work as well as selective agent.

Culture procedure:

Add approximately 10 grams sample to 100 ml of Muller-Kauffmann Tetrathionate Novobiocin Broth. Shake well and place the flask in a water bath for 15 minutes at 45°C. Remove the flask and incubate further at 43°C. Several studies have shown an increased recovery rate of *Salmonella* using selective enrichment at an incubation temperature at 43°C (8). After 18-24 hours and 48 hours incubation the enriched culture is subcultured on Brilliant Green Agar modified (Cat. No. B1801).

Limitation:

This medium is not suitable for the growth of *Salmonella* Typhi, *Salmonella* Sendai, and *Salmonella* Pullorum etc.

Other organisms like *Morganella morganii* and some *Enterobacteriaceae* may grow as well in the medium. Therefore, all presumptive *Salmonella* colonies that are recovered has to be confirmed by a further test.

Cultural characteristics after 18-48 hours at 43°C (with iodine and novobiocin), when subcultured on TSA.

Organisms (ATCC)	Inoculum	Recovery
<i>Escherichia coli</i> (25922)	50-100	-/+
<i>Proteus vulgaris</i> (13315)	50-100	-/+
<i>Shigella flexneri</i> (12022)	≥10 ³	-
<i>Salmonella</i> Enteritidis (13076)	50-100	+++
<i>Salmonella</i> Paratyphi A (9150)	50-100	+++
<i>Salmonella</i> Paratyphi B (8759)	50-100	+++
<i>Salmonella</i> Typhi (6539)	≥10 ³	-
<i>Salmonella</i> Typhimurium (14028)	50-100	+++

References:

1. L. Mueller, C. R. Soc. Biol., (Paris) 89, 434 (1923)
2. F. Kauffman, Ztschr. F. Hyg., 117, 26 (1935)
3. ISO 6579:2002 specifies a horizontal method for the detection of *Salmonella*, including *Salmonella* Typhi and *Salmonella* Paratyphi.
4. Public Health Laboratory Service, Monograph Series No. 8, Public Health Laboratory Service, London, England (1974)
5. R.W.S. Harvey, T.S. Price, J. Hyg. Camb., 77, 333 (1976)
6. L. Jeffries, J. Clin. Pathol., 12, 568 (1959)
7. M.L. Speck, (Ed.), Compendium of Methods for the Microbiological Examination of Foods, 2nd Ed., American Public Health Association, Washington, D.C. (1984)
8. J.Y. D'Aoust, *Salmonella* in Food borne Bacterial pathogens, (Eds.) Doyle M. P., 327, Marcel Dekker, New York (1989)

Precautions and Disclaimer

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