TM 1399 -MULLER KAUFFMAN TETRATHIONATE NOVOBIOCIN BROTH BASE (ISO 6579-1:2017)

INTENDED USE

For enrichment and isolation of *Salmonellae* by suppression *Proteus* species.

PRODUCT SUMMARY AND EXPLANATION

Muller-Kauffman tetrathionate novobiocin broth is used for enrichment and isolation of *Salmonellae* from food and animal feeds. The medium was first described by Muller in (1923) for inhibition of coliform bacteria at the same time as permitting the development of typhoid and paratyphoid bacilli. Kauffman modified the medium's formulation include ox bile and brilliant green as selective agents to suppress bacteria such as *Proteus* species. The selectivity of this medium is conferred by tetrathionate.

COMPOSITION

Ingredients	Gms / Ltr
Sodium thiosulphate, pentahydrate	47.800
Calcium carbonate	38.700
Enzymatic digest of casein	8.60
Ox bile	4.78
Meat Extract	4.30
Sodium chloride	2.60
Brilliant green	0.0096

PRINCIPLE

Medium contains Sodium thiosulphate which is responsible to produce tetrathionate by adding iodine to the culture medium. Tetrathionate suppresses the growth of coliform and other enteric bacteria and most intestinal bacteria. Sodium chloride liberates the essential electrolytes for transport and osmotic balance. Calcium carbonate in the medium acts as a buffer. Brilliant green, Ox bile and Novobiocin supplement inhibits the growth of gram-positive bacteria other than *Salmonella*.

INSTRUCTION FOR USE

- Dissolve 89.42 grams (equivalent weight of dehydrated medium per litre) in 1000 ml distilled water.
- Gently heat to boiling with swirling to dissolve the medium completely. Do Not Autoclave.
- Cool to 45-50°C.
- Aseptically add rehydrated contents of 1 vial of MKTT Novobiocin Supplement (TS 152) and 20 ml of iodine-iodide solution (20 gram iodine and 25 gram potassium iodide in 100 ml sterile distilled water).
- Mix well to dispense into sterile tubes.

Note:

1. Medium should be mixed before dispensing to disperse calcium carbonate uniformly.
2. Due to presence of calcium carbonate, the prepared media forms opalescent solution with white precipitate.

QUALITY CONTROL SPECIFICATIONS

Appearance of Dehydrated powder : Cream to greenish yellow, homogeneous free flowing powder

Appearance of Prepared medium : Light green coloured, opalescent solution with heavy white precipitate.

pH (at 25°C) : 8.0± 0.2

INTERPRETATION

Cultural characteristics observed after incubation with addition of 20ml iodine solution and MKTT Novobiocin Supplement (TS 152). Further subculture is carried out on XLD Agar, Modified (TM 1621) and incubated.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Colour of Colony	Recovery on XLD Agar (TM 1621)	Incubation Temperature*	Incubation Period*
Salmonella enteritidis	13076	50-100	Good-Luxuriant	Red colonies w/ black centre	>=70%	37 ±1°C	24 ± 3 Hours
Escherichia coli	8739	>1000	Partial inhibition		-	37 ±1°C	24 ± 3 Hours
Escherichia coli	25922	>1000	Partial inhibition		-	37 ±1°C	24 ± 3 Hours
Salmonella Typhimurium	14028	50-100	Good-Luxuriant	Red colonies w/ black centre	>=70%	37 ±1°C	24 ± 3 Hours
Enterococcus faecalis	29212	>1000	Inhibition-Partial inhibition		-	37 ±1°C	24 ± 3 Hours

*Incubation condition same after addition of 20ml iodine solution and MKTT Novobiocin Supplement (TS 152) or subculturing on XLD Agar.

SELECTIVITY

Cultural characteristics observed after incubation* with addition of 20ml iodine solution and MKTT Novobiocin Supplement (TS 152). Further subculture is carried out on Tryptone Soya Agar and incubated*.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	CFU on Tryptone Soya Agar
Escherichia coli	8739	>1000	Partial inhibition	<=100 colonies
Escherichia coli	25922	>1000	Partial inhibition	<=100 colonies
Enterococcus faecalis	29212	>1000	Inhibition-Partial inhibition	<=10 colonies
Enterococcus faecalis	19433	>1000	Inhibition-Partial inhibition	<=10 colonies

*Incubation conditions after addition of 20ml iodine solution and MKTT Novobiocin Supplement (TS 152) is 41.5 ±1°C for 24 ± 3 hour. Whereas, after subculturing it is 37 ±1°C for 24 ± 3 hours.

PACKAGING

In 500 gm packaging size.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 25°C and protect from direct Sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

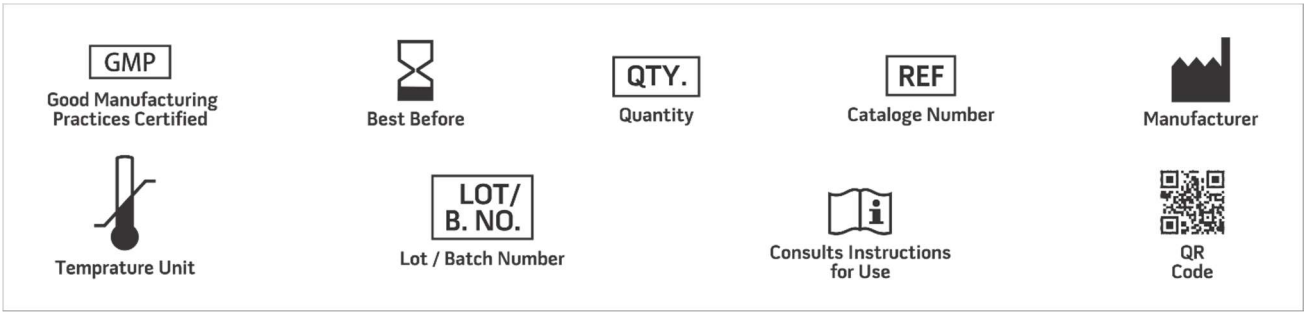
Product Deterioration: Do not use, if powder show evidence of microbial contamination, discoloration, drying, or other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

1. Harvey R. W. S. and Price T. S., 1976, J. Hyg. Camb., 77:333
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1. I
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition
4. Kauffman F., 1935, Ztschr. F. Hyg., 117:26.
5. Public Health Laboratory Service, 1974, Monograph Series No. 8, Public Health Laboratory Service, London, England.
6. Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Detection of Salmonella spp. ISO 6579-1:2017.
7. Mueller L., 1923, C. R. Soc. Biol., (Paris) 89:434.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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