PRODUCT DATA SHEET

MUELLER KAUFFMAN TETRATHIONATE NOVOBIOCIN BROTH BASE
TM 1399

For enrichment and isolation of Salmonellae

Composition

Ingredients	Gms/Ltr.
Calcium carbonate	38.70
Sodium thiosulphate (anhydrous)*	30.45
Enzymatic digest of casein	8.60
Ox bile	4.78
Meat Extract	4.30
Sodium chloride	2.60
Brilliant green	0.0096

* Dehydrated powder, hygroscopic in nature, store in a dry place in tightly- sealed containers 25°C and protected from direct Sunlight.

Instructions for use

Dissolve 89.4gms in 1000ml distilled water. Allow to soak for 10 minutes, swirl to mix and gently heat to boil. DO NOT AUTOCLAVE. Cool to 45^{0}C prior to use. Add 20ml of Iodine - Iodide solution and 1 vial of **MKTT Novobiocin Supplement (TS 152)**. Mix well and distribute into sterile tubes.

Iodine-iodide solution
Dissolve 25gm of potassium iodide in 10ml of water. Add 20gm iodine and dilute to 100ml with sterile deionised water.

Note: Due to presence of calcium carbonate, the prepared media forms opalescent solution with white precipitate. If necessary, adjust the pH to the desired value, following the protocol used.

Appearance: Green turbid solution that precipitates on standing
pH (at 25°C): 8.2 ± 0.2

Principle

MUELLER-KAUFFMANN TETRATHIONATE NOVOBIOCIN BROTH 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. 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 neutralizes the sulphuric acid to produced when tetrathionate is reduced. Brilliant green, Ox bile and Novobiocin supplement inhibits the growth of gram positive bacteria other than *Salmonella*.

PREENRICHMENT and SELECTIVE ENRICHMENT

1. Add 25gm of the sample to 225ml of Buffered Peptone Water (TM 307) and incubate at 37 ± 1°C for 18 ± 2 hours.

2. Transfer 0.1ml of the pre-enrichment culture to 10ml of Rappaport Vassiliadis Soya Broth (TM 1282). Incubate at 41.5°C for 24 ± 3 hours.
3. Cool the medium to 25°C.
4. Transfer 1ml of the pre-enrichment culture to 10ml of Muller Kauffmann Broth Base (MKTTN) including the iodine-iodide solution and 5ml of novobiocin solution as described above. Incubate at 37 ± 1°C for 24 ± 3 hours.
5. Isolate on XLD agar (TM 1621) and on a second selective isolation media, with a platinum loop.
6. Using well formed colonies, inoculate on a nutrient agar also which serves as the starting points for correct morphological identification.

Interpretation

Cultural characteristics observed after inoculating (10^3CFU/ml), on incubation at 37°C for 24 hours at then subcultured onto XLD Agar.

Microorganisms	ATCC	Inoculum (CFU/ml)	Growth
Salmonella tyhphimurium	14028	10^3	≥ 95%
Escherichia coli	25923	10^3	≤ 5%
Proteus mirabilis	29906	10^3	≤ 5%

Reference

1. SO 6579, International Standardization Organization. Microbiology of Food and animal feeding stuffs - Horizontal method for the detection of Salmonella spp. EDEL, W., a. KAMPELMACHER, E.H. (1969): Salmonella isolation in nine European laboratories using a standardized technique. - Bull. Wld. Hlth. Org., 41; 297-306. (2002).
2. MÜLLER, L. Un nouveau milieu d'enrichissement pour la recherche du bacille typhique et des paratyphiques. Comptes Rendus de la Société de Biologie, 8: 434-437. (1923).
3. KAUFFMANN, F. Weitere Erfahrungen mit dem kombinierten Anreicherungsverfahren für Salmonella bazillen. Zeitschrift für Hygiene und Infektionskrankheit, 11: 26-32. (1935).