MUELLER KAUFFMANN BROTH BASE w/BRILLIANT GREEN & NOVOBIOCIN (MKTTN) ISO 6579

CAT N°: 1173   For the selective enrichment of *Salmonella*

FORMULA IN g/l

Calcium Carbonate	38.70	Beef Extract	4.30
*Sodium Thiosulfate Anhydrous	30.5	Sodium Chloride	2.60
Enzymatic Digest of Casein	8.60	Novobiocin	0.04
Ox Bile	4.78	Brilliant Green	0.0096
* Equivalent to 47.8 gr of Sodium Thiosulfate pentahydrated			

Final pH 8.0 ± 0.2 at 25°C

PREPARATION

Suspend 89.53 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE.Cool to 45-50°C. Aseptically Add 20 ml of a iodine and potassium iodide solution (20 g of iodine and 25 g of potassium iodide in 100 ml of sterile distilled water). Homogenize gently and dispense into sterile containers. The prepared medium should be stored at 2-8°C. The color is green-blue.

The dehydrated medium should be homogeneous, free-flowing and white in color. If there are any physical changes, discard the medium.

USES

MULLER-KAUFFMANN BROTH BASE WITH BRILLIANT GREEN & NOVOBIOCIN (MKTTN) is recommended by the ISO 6579 norm to be used as a selective enrichment broth for the detection of *Salmonella spp* in all food types, including milk and dairy products, molluscan shellfish and other fish products, and in environmental swabs.

Beef extract and Casein peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Calcium carbonate is a neutralizer which absorbs toxic metabolites. Bile salts, Brilliant green and Novobiocin inhibit organisms other than *Salmonella*. Selectivity is also obtained by both sodium thiosulfate and tetrathionate, suppressing coliforms. Tetrathionate is formed in the medium with the addition of the iodine and potassium iodide solution. Organisms containing the enzyme tetrathionate reductase will thrive in this medium. Sodium chloride supplies essential electrolytes for transport and osmotic balance.

PREENRICHMENT and SELECTIVE ENRICHMENT

1. Add 25 g of the sample to 225 ml of Buffered Peptone Water ISO 6579 (Cat.1402) and incubate at 37±1°C for 18 ± 2 hours.
2. Transfer 0.1 ml of the preenrichment culture to 10 ml of Rappaport Vassiliadis Soy Broth (Cat. 1174). Incubate at 41.5°C for 24 ± 3 hours.
3. Transfer 1 ml of the preenrichment culture to 10 ml of Muller Kauffmann Broth Base (MKTTN). Incubate at 37 ± 1°C for 24 ± 3 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 37 ± 1 °C and observed after 24 ± 3 hours.

Microorganisms	Inoculum Concentration	Growth	
		6 hours	24 hours
Escherichia coli ATCC 25922	99%	< 30%	< 5%
Salmonella typhimurium ATCC 14028	1%	> 70%	> 95%

According ISO 11133 24h/37 °C (Productivity and Selectivity)

Microorganisms	Inoculum (cfu/ml)	Productivity Semi quantitative	Selectiviity Semi quantitative	Specificity
Salmonella typhimurium ATCC 14028	$10^3 / 10^4$	> 10 colonies		red with black center
Escherichia coli ATCC 8739	$10^4 / 10^6$		Inhibited	
Enterococcus faecalis ATCC 29212	$10^4 / 10^6$		<10 colonies	

Media Productivity.- (XLD)
Media Selectivity.- (TSA)

BIBLIOGRAPHY

ISO 6579 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella spp*

Kauffmann, F. 1935. Weitere erfahrungen mit dem kombininierten anreicherungsverfahren fur *Salmonella* bazillen. Ztschr. F. Hyg. 117: 26-32.

ISO

STORAGE

Once opened keep powdered medium closed to avoid hydration.

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