Muller Kaufmann Broth Base with brilliant Green and Novobiocin (MKTTN) ISO

Cat. 1173

For the selective enrichment of Salmonella.

Practical information

Aplications	Categories
Enrichment	Salmonella

Industry: Water / Food

Regulations: ISO 11133 / ISO 19250 / ISO 6579

Principles and uses

Muller-Kauffmann Broth Base with Brilliant Green and Novobiocin (MKTTN) is recommended by the ISO 6579 and ISO 19250 norms 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 water samples and 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.

Formula in g/L

Enzymatic digest of casein	8,6	Beef extract	4,3
Brilliant green	0,0096	Calcium carbonate	38,7
Novobiocin	0,04	Ox Bile	4,78
Sodium chloride	2,6	Sodium thiosulfate	30,5

Preparation

Suspend 89,53 grams of the medium in one litre 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 45-50ºC. Aseptically Add 20 ml of an 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.

Instructions for use

* For detection of Salmonella spp. in food, animal feed, animal faeces, and environmental samples according to ISO 6579:

- Preenrichment in non-selective liquid medium:
Inoculate the Buffered Peptone Water (Cat. 1402) with the sample or dilutions, and incubate at 34-38 ºC for 18 h.
- Enrichment in/on selective media:
Inoculate, with the culture obtained in the pre-enrichment stage, the Rappaport Soy Broth (Vassiliadis)(Cat. 1174) or the Modified Semisolid Rappaport Vassiliadis medium (MSRV) (Cat. 1376), and the MKKTN Broth(Cat. 1173).
The Rappaport Soy Broth and the Modified Semisolid Rappaport medium are incubated at 41,5 ºC for 24 h, and the MKKTN Broth at 37 ºC for 24 h.
- Plating out on selective solid media:
From the selective enriched cultures, inoculate two selective isolation agar; XLD agar (Cat. 1274) and any other selective medium complementary to XLD agar (Salmonella Chromogenic Agar (Cat. 1122), Brilliant Green Agar (Cat. 1143), Bismuth Sulfite Agar (Cat. 1011), DCLS Agar(Cat. 1045), Desoxycholate Citrate Agar (Cat. 1067), Hektoen Enteric Agar (Cat. 1030), Salmonella Shigella Agar(Cat. 1064) and XLT4 Agar (Cat. 1159)).
Incubate the XLD plates inverted at 37 ºC for 24±3 h.
Incubate the second selective medium in accordance with the manufacturer's instructions.
- Confirmation:
Subculture colonies of presumptive Salmonella and confirm their identity by biochemicals and serological tests.

* For detection of Salmonella spp. in water samples according to ISO 19250:

- Preenrichment in non-selective medium:
Inoculate the Buffered Peptone Water (Cat. 1402) with the sample or dilutions, and incubate at 36±2 ºC for 18±2 h.
- Enrichment in selective media:
Inoculate, with the culture obtained in the pre-enrichment stage, the Rappaport Soy Broth (Vassiliadis)(Cat. 1174) and the MKKTN Broth (Cat. 1173).
The Rappaport Soy Broth is incubated at 41,5±1 ºC and the MKKTN Broth at 37±1 ºC, both of them for 24±3 hours.
- Plating out on selective solid media:
From the selective enriched cultures, inoculate two selective isolation agar; XLD agar (Cat. 1274) and any other selective medium complementary to XLD agar (For instance, Brilliant Green Agar (Cat. 1143) or Bismuth Sulfite Agar (Cat. 1011))
Incubate the XLD plates inverted at 36±2 ºC for 24±3 hours.
Incubate the second selective medium in accordance with the manufacturer's instructions.
- Confirmation:
Subculture colonies of presumptive Salmonella and confirm their identity by biochemicals and serological tests.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	White	Green-blue	8,0 ± 0,2

Microbiological test

According to ISO 11133:
Incubation conditions: (37±1 ºC /24±3 h).
Inoculation conditions: Target microorganisms (<100 CFU) / Non-target microorganism (>1000 CFU) / Selectivity (10^4-10^6 CFU).

Microrganisms	Specification	Characteristic reaction
Salmonella typhimurium ATCC 14028 +Escherichia coli ATCC 8739 +Pseudomonas aeruginosa ATCC 27853	> 10 colonies on XLD or other medium of	Colonies with black centre and a light transparent zone of reddish colour due to the colour change of the medium
Salmonella enteriditis ATCC 13076 +Escherichia coli ATCC 8739 +Pseudomonas aeruginosa ATCC 27853	> 10 colonies on XLD or other medium of	Colonies with black centre and a light transparent zone of reddish colour due to the colour change of the medium
Enterococcus faecalis ATCC 29212	< 10 colonies on TSA	
Escherichia coli ATCC 8739	Partial inhibition <100 colonies on TSA	

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

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 19250 water quality-detection of Salmonella spp