Product :
BOLTON ENRICHMENT BROTH BASE

Specification

Liquid culture medium used for the enrichment of *Campylobacter* from food samples according to the ISO standard.

Formula * in g/L

Meat peptone..... 10.00
Lactalbumin hidrolysate..... 5.00
Yeast extract..... 5.00
Sodium chloride..... 5.00
Sodium pyruvate..... 0.50
Sodium metabisulfite..... 0.50
Sodium carbonate..... 0.60
a-Ketoglutaric acid..... 1.00
Haemin..... 0.01

Final pH 7,4 ±0,2 at 25 ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 13,8 g of the powder in 475 mL of distilled water, heating if necessary. Sterilize in the autoclave at 121ºC for 15 minutes. Cool to 47-50º C, add 25 mL of lysed horse blood aseptically, and the content of one vial of Campylobacter Bolton Selective Supplement (Art. No.06-131LYO1). Mix throughly. Dispense the complete medium into suitable containers.

Note: If the enrichment broth has been prepared in advance, it should be kept for no more than 4 hours at ambient temperature or in the dark at 3 ± 2ºC for not more than 7 days.

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Description

Bolton Broth Base is intended for the enrichment of *Campylobacter* from food samples. Food processing and preservation injure *Campylobacter* cells and resuscitation steps by a double incubation in Bolton Broth encourages them to multiply and grow.
The meat peptone and lactalbumin hydrolysate supply the carbon and nitrogen for growth. Sodium chloride provides osmotic balance and the sodium carbonate neutralizes the acidity generated by the microbial growth. Yeast extract and ketoglutaric acid act as growth factors. Inclusion of sodium metabisulfite, sodium pyruvate and haemin neutralises toxic compounds that may form in the culture medium due to the action of oxygen action and avoid the need for a microaerobic atmosphere. Horse Lysed blood is necessary to neutralize trimethoprim antagonists present in the medium.
The selectivity of the enrichment step is optimized with the Selective Supplement: Vancomycin is active against Gram positive microorganisms. Cephoperazone is predominantly active against Gram negative bacteria. Trimethoprim acts against a wide variety of Gram positive and Gram negative cells and cycloheximide or amphotericin B are efficient fungicides.

Necessary supplements

Campylobacter Bolton Selective Supplement (Art. No 06-131LYO1)
Vial Contents:
Necessary amount for 500 mL of complete medium.
Vancomycin 10.0 mg
Cefoperazone 10.0 mg
Trimethoprim 10.0 mg
Cycloheximide 25.0 mg

Distilled water (Solvent)

Campylobacter Bolton Selective Supplement (Art. No 06-791LYO1)
Vial Contents:
Necessary amount for 250 mL of complete medium.
Vancomycin 5.0 mg
Cefoperazone 5.0 mg
Trimethoprim 5.0 mg
Cycloheximide 12.5 mg

Distilled water (Solvent)

Technique

Introduce a quantity (mass or volume) into nine times its volume of Bolton Selective Enrichment Broth so as to obtain a test sample/medium ratio of 1:10 (w/v or v/v) and homogenize.
Bolton Selective Enrichment Broth does not require incubation in a microaerobic envferment, but must be used in screw topped containers which are filled leaving a headspace of less than 20 mm, and have tightly closing caps.
Incubate the initial suspension à 37ºC for 4-6 hours, then à 41,5ºC for 44 ± 4 hours.
For the isolation and identification techniques, please, refer to ISO or BAM (Bacteriological Analytical Manual) methods.

Quality control

Incubation temperature: 37ºC ±1 / 41,5ºC ±1 **Incubation time:** 5 h± 1 / 44 ±4 h

Inoculum: Practical range 100 ± 20 CFU. Min. 50 CFU (Productivity) / 10^4-10^6 CFU (Selectivity) according to ISO 11133:2014/Amd 1:2018. Microaerobic atmosphera

Microorganism	Growth	Remarks
Campylobacter jejuni ATCC® 29428	Good recovery in CCD ≥ 10 CFU	Grey-flat-humid colonies, sometimes metallic shine
Escherichia coli ATCC® 8739	Inhibited in TSA	-
Proteus mirabilis ATCC® 29906	Inhibited in TSA	-
ATCC® 29428 + 8739 +29906	Good recovery in CCD ≥ 10 CFU	Grey-flat-humid colonies, sometimes metallic shine

References

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· ISO 10272-1 Standard (2017) Microbiology of the food chain - Horizontal Method for detection and enumeration of Campylobacter spp. - Part 1: Detection method.

· ISO 10272-2 Standard (2017) Microbiology of the food chain - Horizontal Method for detection and enumeration of Campylobacter spp. - Part 2:Colony count-tecnique.

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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).