Technical Data sheet

GranuCult
BOLTON Broth (Base)
acc. ISO 10272

Ordering number: 1.00068.0500

For the selective enrichment of Campylobacter spp. from food and animal feed as well as from environmental samples and other materials.

This culture medium complies with the specifications given by EN ISO 10272 and FDA-

BAM.

Mode of Action

This medium is designated especially to improve the resuscitation of sublethally injured

Camplyobacter.

Cefoperazone, vancomycin, cycloheximide and trimethoprim are the selective agents used to inhibit the accompanying gram-positive and gram-negative bacteria as well as yeasts and moulds.

Typical Composition

Specified by EN ISO 10272-1		**FDA-BAM M28a**		**GranuCult™ BOLTON Broth acc. ISO 10272**	
Enzymatic Digest of Animal Tissues	10 g/l	Meat Peptone	10 g/l	Enzymatic Digest of Animal Tissues*	10 g/l
Lactalbumin Hydrolysate	5 g/l	Lactalbumin Hydrolysate	5 g/l	Lactalbumin Hydrolysate	5 g/l
Yeast Extract	5 g/l	Yeast Extract	5 g/l	Yeast Extract	5 g/l
Sodium Chloride	5 g/l	Sodium Chloride	5 g/l	Sodium Chloride	5 g/l
α-Ketoglutaric Acid	1 g/l	α -Ketoglutaric Acid	1 g/l	α-Ketoglutaric Acid**	1 g/l
Sodium Pyruvate	0.5 g/l	Sodium Pyruvate	0.5 g/l	Sodium Pyruvate	0.5 g/l
Sodium Metabisulphite	0.5 g/l	Sodium Metabisulphite	0.5 g/l	Sodium Disulfite***	0.5 g/l
Sodium Carbonate	0.6 g/l	Sodium Carbonate	0.6 g/l	Sodium Carbonate	0.6 g/l
Haemin	0.01 g/l	Haemin	0.01 g/l	Haemin	0.01 g/l
Water	1000 ml/l	Water	1000 ml/l	Water	n/a
pH at 25 °C	7.4 ± 0.2	pH at 25 °C	7.4 ± 0.2	pH at 25 °C	7.4 ± 0.2

upplements to be added after autoclaving					
ancomycin	0.02 g/l	ancomycin	0.02 g/l	Vancomycin	0.02 g/l
Cefoperazone	0.02 g/l	Cefoperazone	0.02 g/l	Cefoperazone	0.02 g/l
Trimethoprim lactate	0.02 g/l	Trimethoprim lactate	0.02 g/l	Trimethoprim lactate	0.02 g/l
Amphotericin B	0.01 g/l	Amphotericin B	0.01 g/l	Amphotericin B	0.01 g/l
Lysed Horse Blood	50 ml/l	Lysed Horse Blood	50 ml/l	Lysed Horse Blood	50 ml/l

* Following EN ISO 11133, the term enzymatic digest of animal tissues includes meat peptone.

** α -Ketoglutamic Acid is e uivalent to α-Ketoglutaric acid.

***Sodium disulfite is e uivalent to sodium metabisulphite.

Preparation

Dissolve 27.6 g in 1 l of purified water and autoclave 15 minutes at 121°C. Cool to 45-50°C. Aseptically add 50 ml lysed horse blood and the dissolved content of 2 vials of Bolton Broth Selective Supplement (article number 1.00079.0010).

The prepared medium is dark red to black.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

According to EN ISO 10272, the test portion is inoculated into the Bolton Selective Enrichment Broth and homogenized.

It is incubated in a microaerobic atmosphere at 36-38°C for 4-6 h and then at 40.5-42.5°C for 40-48 h. Microaerobic atmosphere can be achieved by using Merck Anaerocult® C or C mini in an anaerobic jar.

From the culture obtained in Bolton Selective Enrichment Broth, two selective solid media are inoculated: mCCD agar (GranuCult™ mCCD Agar acc. ISO 10272, article number 1.00070.0500) and another solid selective medium based on a principle different from that of mCCD agar.

Plates must be dried directly prior to inoculation in order to prevent presence of condensing water on the surface and swarming of the bacteria (see EN ISO 11133).

Incubate the solid media in a microaerobic atmosphere at 40.5-42.5°C for 40-48 h to detect the presence of colonies presumed because of their characteristics to be *Campylobacter*.

Prevent drying out of the surface of the plates during incubation!

These colonies are confirmed following the procedure given by EN ISO 10272.

Direct screening for *Campylobacter jejuni* and *Campylobacter coli* from the enrichment in Bolton Selective Enrichment Broth is possible using Singlepath® Campylobacter (article number 1.04143.0001).

Storage

Store at +15°C to +25°C, dry and tightly closed. Do not use clumped or discolored medium. Protect from U light (including sun light). For *in vitro* use only.

According to EN ISO 10272-1, self-prepared complete medium can be stored at +1°C to +5°C in the dark and protected against evaporation for up to 7 days.

Quality Control

Function	Control strains	Incubation	Method of control	Expected results
Productivity	*Campylobacter coli* ATCC® 43478 + *Escherichia coli* ATCC® 25922 + *Proteus mirabilis* ATCC® 29906	4-6 h at 36-38 °C, afterwards 40-48 h at 40.5-42.5°C microaerobic	Qualitative	>10 colonies on GranuCult™ mCCD Agar acc. ISO 10272, greyish colonies
	Campylobacter jejuni ATCC® 33291 + *Escherichia coli* ATCC® 25922 + *Proteus mirabilis* ATCC® 29906			
	Campylobacter jejuni ATCC® 29428 + *Escherichia coli* ATCC® 8739 + *Proteus mirabilis* ATCC® 29906			
Selectivity	*Escherichia coli* ATCC® 8739	4-6 h at 36-38 °C, afterwards 40-48 h at 40.5-42.5°C microaerobic	Qualitative	No growth on Tryptic Soy Agar (TSA)
	Escherichia coli ATCC® 25922			
	Proteus mirabilis ATCC® 29906			

Please refer to the actual batch related Certificate of Analysis.

The performance test is in accordance with the current version of EN ISO 11133.

Literature

Bayliss, C.L., McPhee, S., Martin, K.W., Humphrey, T.J. and Betts, R.P. (2000): Comparison of three enrichment media for the isolation of *Campylobacter spp.* from foods. J. Appl. Microbiol. **89**: 884-891.

Corry J.E.L. and Atabay, H.I. (2012): Culture media for the isolation of *Campylobacters*, *Helicobacters* and *Arcobacters*. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds)., pp. 403 - 435. Royal Society of Chemistry, Cambridge, UK.

FDA-BAM (2001): Chapter No. 7: *Campylobacter.* U.S. Food and Drug Administration - Bacteriological Analytical Manual.

ISO International Standardisation Organisation. Microbiology of food, animal feed and water - Horizontal method for detection and enumeration of Campylobacter spp. - Part 1: Detection method. EN ISO 10272-1:2006.

ISO International Standardisation Organisation. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media. EN ISO 11133:2014.

Musgrove, M.T., Berrang, M.E., Byrd, J.A., Stern, N.J. and Cox, N.A. (2001): Detection of *Campylobacter spp*. in ceca and crops with and without enrichment. Poult. Sci. **80**: 825-828.

Willis, W.L., Murry, C. and Talbott, C. (2000): Effect of delayed placement on the incidence of *Campylocter jejuni* in broiler chicken. Poult. Sci. **79**: 1392-1395.

Ordering Information

Product	Cat. No.	Pack size
GranuCult™ BOLTON Broth (Base) acc. ISO 10272	1.00068.0500	500 g
Bolton Broth Selective Supplement acc. ISO 10272	1.00079.0010	10 vials
GranuCult™ mCCD (Modified Charcoal Cefoperazone Deoxycholate) Agar (Base) acc. ISO 10272	1.00070.0500	500 g
CCDA Selective Supplement	1.00071.0010	10 vials
Anaerobic jar	1.16387.0001	1 piece
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® C	1.16275.0001	1 x 10
Anaerocult® C mini	1.13682.0001	1 x 25
Plate basket	1.07040.0001	1 piece
Singlepath® Campylobacter	1.04143.0001	25 tests

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