Technical Data Sheet

GranuCult®
BAT broth acc. IFU Method No. 12

Ordering number: 1.07993.0500

For the enrichment of spore-forming thermo-acidophilic spoilage bacteria (*Alicyclobacillus* spp.) from juice and juice-related products and their ingredients, environmental samples including process water and other materials in the area of food production and food handling.

This culture medium complies with the specifications given by IFU Method No. 12.

BAT broth is named from *Bacillus acidoterrestris* (BAT), the former name of *Alicyclobacillus acitoterrestris*.

Mode of Action

BAT broth contains glucose as a carbon and energy source. Yeast extract is source for vitamins, particularly the B-group. Potassium dihydrogen phosphate acts as a buffering system. The low pH value and the high incubation temperature inhibit the contaminating flora. This medium contains many trace elements which supply the specific requirements of the spore-forming thermo-acidophilic spoilage bacteria (*Alicyclobacillus* spp.).

Following the latest revision of IFU method No. 12 of 2019, the toxic components cobalt(II) chloride hexahydrate and boric acid have been removed from the formulation.

Typical Composition

Specified by IFU Method No. 12		**GranuCult® BAT broth acc. IFU Method No. 12**	
Yeast extract	2 g/l	Yeast extract	2 g/l
Glucose anhydrous	5 g/l	D(+) glucose	5 g/l
Calcium chloride dihydrate	0,25066 g/l	Calcium chloride	0.25066 g/l
Magnesium sulfate heptahydrate	0,5 g/l	Magnesium sulfate	0.5 g/l
Ammonium sulfate	0,2 g/l	Ammonium sulfate	0.2 g/l
Potassium dihydrogen phosphate	3,0 g/l	Potassium dihydrogen phosphate	3 g/l
Zinc sulfate monohydrate	0,00018 g/l	Zinc sulfate	0.00018 g/l
Copper sulfate pentahydrate	0,00016 g/l	Copper sulfate	0.00016 g/l
Manganese sulfate hydrate	0,00015 g/l	Manganese sulfate	0.00015 g/l
Sodium molybdate dihydrate	0,00030 g/l	Sodium molybdate	0.00030 g/l
Water	1000 ml/l	Water	n/a
pH at 25 °C	4.0 ± 0.2	pH at 25 °C	4.0 ± 0.2

Preparation

Dissolve 11.0 g in liter of purified water, adjust the pH to 4.0 ± 0.2 by adding 1N H_2SO_4 and autoclave (15 min. at 121 °C).

The prepared medium is clear and yellowish.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Following the procedure for detection given by IFU Method No. 12, prepare the sample and dilute it using BAT broth, followed by the heat treatment at 80 °C for 10 min.

Incubate for 5 d at 45 °C ± 1 °C aerobically.

Disperse 0,1 ml of the incubated enrichment onto the surface of a plate containing GranuCult® BAT agar (article number 1.070094.0500) or alternatively using 1 ml of the enrichment with poured plate method using BAT agar.

Incubate 48 h ± 4 h at 45 °C ± 1 °C aerobically. If growth occurs, proceed with confirmation. If there is no growth after 48 h ± 4 h, return the plate for further incubation at 45 °C ± 1 °C aerobically for additional 72 ± 4 h.

Examine the plates for the presence of colonies.

BAT media support the growth of all currently known species of *Alicyclobacillus* spp. It is therefore likely that a wider range of colony types will be visible on these media.

For the confirmation of presumptive *Alicyclobacillus* spp. from the detection procedure take at least one colony. One confirmed isolate per sample is sufficient for the detection procedure. If the first colony is negative, take further colonies up to a maximum of five colonies.

From each selected colony, streak a portion onto one plate containing a culture medium with neutral pH, e.g. GranuCult® Plate Count agar (article number 1.05463.0500), and one plate containing GranuCult® BAT agar (article number 1.070093.0500).

Incubate both plates for 72 h ± 4 h at 45°C ± 1 °C aerobically.

Spore-forming thermo-acidophilic bacteria (*Alicyclobacillus* spp.) **negative** isolates will show growth on both culture media.

Spore-forming thermo-acidophilic bacteria (*Alicyclobacillus* spp.) **positive** isolates will show growth only on the BAT agar and no growth on the culture medium with the neutral pH, e.g. Plate Count agar.

Storage

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

According IFU Method No. 12, self-prepared medium can be stored in closed containers at 5 °C ± 3 °C in the dark for up to 4 weeks.

Quality Control

Function	Control strains	Incubation	Method of control	Expected results
Productivity	*Alicyclobacillus acidoterrestris* DSM 2498	up to 5 days at 45 ± 1 °C, aerobic	Qualitative single tube method (turbidity)	Growth good to very good
	Alicyclobacillus acidoterrestris DSM 3922			
	Alicyclobacillus cycloheptanicus DSM 4006			
	Alicyclobacillus hesperidium DSM 12766			

Please refer to the actual batch related Certificate of Analysis.

The performance test is in accordance with the current version of IFU Method No. 12 and EN ISO 11133.

Literature

IFU International Fruit and Vegetable Juice Association. Method on the detection and enumeration of spore-forming thermo-acidophilic spoilage bacteria (*Alicyclobacillus* spp.). Method of Analysis No. 12:2019.

ISO International Standardisation Organisation. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media. EN ISO 11133:2014+Amd1:2018.

Deinhard, D., Blanz P., Pooralla K. and Alatan, E. (1987) *Bacillus acidoterrrestris* sp. nov., a new thermotolerant acidophile isolated from different soils. system. Appl. Microbiol. **10** (1), 47-53.

Fritze, D. and Pukall, R. (2012): Culture media for *Bacillus* spp. and related Genera Relevant to Foods. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds). pp. 90 – 114. Royal Society of Chemistry, Cambridge, UK.

Smit, Y., Cameron, M., Venter, P. and Witthuhn, R.C. (2011) *Alicyclobacillus* spoilage and isolation — A review. Food Microbiol. **28** (3), 331-349.

Steyn, C.E., Cameron, M. and Witthuhn, R.C. (2011) Occurrence of *Alicyclobacillus* in the fruit processing environment — A review. Int. J. Food Microbiol. **147** (1), 1-11.

Yokota, A., Fuji T., and Goto K. (eds) *Alicyclobacillus*: Thermophilic Acidophilic Bacilli. Springer, Japan, First Edition, 2007.

Ordering Information

Product	Cat. No.	Pack size
GranuCult® BAT broth acc. IFU Method No. 12	1079930500	500 g
GranuCult® BAT agar acc. IFU Method No. 12	1079940500	500 g
GranuCult® Plate Count agar acc. ISO 4833, ISO 17410 and FDA-BAM	1054630500	500 g
ReadyPlate™ 55 Plate Count Agar	1467630020	20 x 55 mm plates
Cellulose mixed ester filter: S-Pak® filters 0,45 µm, 47 mm, white gridded	HAWG047S6	600 individually sealed filters, sterile
Cellulose mixed ester filter: EZ-Pak® filters 0,45 µm, 47 mm, white gridded	EZHAWG474	4 bands of 150 sterile filters
EZ-Stream® Vacuum Pump	EZSTREAM1	
EZ-Fit® Manifold	EZFITBASE 1, 3, 6	
EZ-Pak® Dispenser Curve	EZCURVE01	