

Technical Data Sheet

GranuCult® BAT agar according IFU Method No. 12

Ordering number: 1.07994.0500

For the enumeration, detection and identification 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 agar is named from *Bacillus acidoterrestris* (BAT), the former name of *Alicyclobacillus acidoterrestris*.

Mode of Action

BAT agar 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.). Agar is the solidifying agent; because of the low pH the gel strength is weak.

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 agar 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

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Sodium molybdate dihydrate	0,00030 g/l	Sodium molybdate	0.00030 g/l
Agar	15 g to 22 g	Agar-Agar*	18 g/l

* Agar-Agar is equivalent to other different terms of agar

Preparation

Dissolve 29.0 g in 1 liter of purified water. Heat in boiling water and agitate frequently until completely dissolved. Autoclave (15 minutes at 121°C).

Note: the medium has a spontaneous pH of 5.3 ± 0.2 to maintain the gel strength during autoclavation.

Cool to 45 -50°C. Adjust the pH to 4.0 ± 0.2 by adding 1N H₂SO₄. Mix well and pour plates.

Use the molten agar as soon as possible and avoid heating or re-melting after the pH adjustment. For pour plate method, the medium may be hold at 44 °C to 47 °C in a water bath, it should not be retained for more than 2 h.

For surface inoculation methods, pour 15 to 20 ml into sterile Petri dishes with small size (90 mm) or pour 90 to 100 ml into sterile Petri dishes with large size (140 mm).

Immediately before use, if necessary, agar plates may be dried following the procedure as described by EN ISO 11133.

The prepared medium is opalescent to turbid 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 GranuCult® BAT broth (article number 1.070093.0500), followed by the heat treatment at 80 °C for 10 min.

For the enumeration procedure by pour plate technique as given by IFU Method No. 12, completely melt the medium BAT agar, then cool it to between 44 °C to 47 °C in a water bath, then adjust the pH (see "Preparation") and use the tempered molten BAT agar as soon as possible.

Mix the cooled sample by swirling and then inoculate by pipetting 10 ml of the diluted sample directly into a sterile large Petri dish (140 mm). Subsequently add the tempered BAT agar to a depth of approximately 3 mm by using a minimum of approximately 90 ml of melted BAT agar. Carefully mix the inoculum with the culture medium by using the *pour plate technique* (8-shape movements) by gently swirling the Petri dish by alternate rotation. Leave for solidification by placing the Petri dish on a cool horizontal surface.

Instead of using one large Petri dish (140 mm) for a sample of 10 ml, five small Petri dishes (90 mm) each with 2 ml sample can be used, filled with 15 – 20 ml tempered molten BAT agar.

After complete solidification, invert the thus prepared plates and place them with bottom up into a plastic bag.

Incubate for 5 d at 45 °C $\pm$ 1 °C aerobically and count the colonies.

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For the enumeration procedure by membrane filtration as given by IFU Method No. 12, mix the cooled sample by swirling and filter 100 ml through a 0,45 µm pore size membrane filter (cellulose ester filters, gridded, 0.45 µm pore size, EZ-Pak®, article number EZHAWG474).

After filtration, clean the sides of the filter with approximately 20 ml of sterile water.

Water samples (e.g. process water samples) need no dilution.

Place the membrane filter onto a pre-poured small sized plate (90 mm) with BAT agar and ensure that there is no air trapped underneath.

Invert the thus prepared plates and place them with bottom up into a plastic bag

Incubate for 5 d at 45 °C ± 1 °C aerobically and count the 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 enumeration procedure take five colonies from each Petri dish, representing each dilution.

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 BAT agar.

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 to IFU Method No. 12, self-prepared plates for the surface inoculation methods, prior to drying, at 5 °C ± 3 °C for up to 14 days.

According to IFU Method No. 12, the base medium may be stored before adjustment of the pH in the dark at 5 °C ± 3 °C for no longer than 3 months, under conditions which do not allow any change in its composition and properties, see EN ISO 11133. Before beginning of the microbiological examination, completely melt the medium, then cool it to 44 °C to 47 °C in a water bath, then adjust the pH (see above) and use the molten agar as soon as possible.

Quality Control

Function	Control strains	Incubation	Method of control	Expected results
Productivity	<i>Alicyclobacillus acidoterrestris</i> DSM 2498	up to 5 days at 45 ± 1 °C, aerobic	Quantitative method for solid media (poured plate)	Recovery ≥ 70 %
	<i>Alicyclobacillus acidoterrestris</i> DSM 3922			
	<i>Alicyclobacillus cycloheptanicus</i> DSM 4006			
	<i>Alicyclobacillus hesperidium</i> DSM 12766			
Productivity	<i>Alicyclobacillus acidoterrestris</i> DSM 2498	up to 5 days at 45 ± 1 °C, aerobic	Quantitative method for solid media (membrane filtration)	Recovery ≥ 70 %
	<i>Alicyclobacillus acidoterrestris</i> DSM 3922			
Selectivity	<i>Bacillus subtilis</i> ATCC® 6633 [WDCM 00003]	up to 5 days at 45 ± 1 °C, aerobic	Qualitative method for solid media (streaking method)	Total inhibition (0)
Selectivity	<i>Bacillus subtilis</i> ATCC® 6633 [WDCM 00003]	up to 5 days at 45 ± 1 °C, aerobic	Qualitative method for solid media (membrane filtration)	Total inhibition (0)

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 using Merck Millipore mixed cellulose ester filters (0.45 µm pore size).

A recovery rate of 70 % is equivalent to a productivity value of 0.7.



Alicyclobacillus acidoterrestris DSM 2498 on BAT agar

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 acidoterrestris* 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 agar acc. IFU Method No. 12	1079940500	500 g
GranuCult® BAT broth acc. IFU Method No. 12	1079930500	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	