

Technical Data Sheet

GranuCult™ CSB (Cronobacter Selective Broth) base acc. ISO 22964

Ordering number: 1.20597.0500

For the selective enrichment of *Cronobacter* spp. from food products and ingredients intended for human consumption and the feeding of animals and environmental samples in the area of food and food.

This culture medium complies with the specifications given by EN ISO 22964:2017.

Mode of Action

The high nutrient content with enzymatic digest of animal tissue and meat extract creates good growth conditions for *Cronobacter*. Vancomycin inhibits the growth of gram-positive flora, sodium chloride maintains the osmotic balance of the medium.

CSB was originally designed as differential broth based on sucrose fermentation, *Cronobacter* mostly produce a color change of the bromocresol indicator. However, this reaction is not always positive for *Cronobacter* in the given incubation time. EN ISO 22964 describes that every selective enrichment in CSB has to be subcultured on CCI (Chromogenic Cronobacter Isolation) agar regardless of its color change.

Typical Composition

Specified by ISO 22964:2017		GranuCult® CSB (Cronobacter Selective Broth) base acc. ISO 22964	
Enzymatic digest of animal tissues	10 g/l	Enzymatic digest of animal tissues	10 g/l
Meat Extract	3 g/l	Meat Extract	3 g/l
NaCl	5 g/l	NaCl	5 g/l
Bromocresol purple	0.04 g/l	Bromocresol purple	0.04 g/l
Sucrose	10 g/l	Surcrose	10 g/l
Supplements added after heating			
Vancomycin hydrochloride	0.01 g/l	Vancomycin hydrochloride	0.01 g/l
Water	1000 ml/l	Water	n/a
pH at 25 °C	7.4 ± 0.2	pH at 25 °C	7.4 ± 0.2



Preparation

Dissolve 14.02 g in 500 ml of purified water. Distribute 10 ml portions into tubes. Autoclave (15 minutes at 121°C).

Dissolve the contents of one vial CSB selective supplement, Cat. No. 120595, in 1 ml of sterile purified water. Add to each tube base broth 0.02 ml (20 µl) of solved CSB selective supplement after it has cooled to below 50°C.

The final concentration is 10 mg vancomycin per liter of CSB.

1 vial CSB selective supplement, Cat. No. 120595, contains 5.0 mg vancomycin hydrochloride.

The prepared medium is clear and purple.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Following the procedure given by EN ISO 22964, after incubation of the inoculated pre-enrichment medium (Buffered peptone water), mix well and transfer 0,1 ml of the obtained culture into 10 ml CSB and mix well.

Incubate the inoculated tubes under aerobic conditions, e.g. acc. to EN ISO 22964 at 40,5-42,5 °C for 22-26 h.

From the culture obtained in the selective enrichment, inoculate the surface of CCI (Chromogenic Cronobacter Isolation) agar, see details given by EN ISO 22964.

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 EN ISO 22964, self-prepared basal medium can be stored in closed containers at +2 °C to +8 °C in the dark and protected against evaporation for up to 6 months.

According EN ISO 22964, self-prepared complete medium may be kept at +2 °C to +8 °C for 1 day.

Quality Control

Function	Control strains	Incubation	Method of control	Criteria	Expected results
Productivity	<i>Cronobacter sakazakii</i> ATCC® 29544 [WDCM 00214] + <i>Staphylococcus aureus</i> ATCC® 6538 [WDCM 00032]	22-26 h at 40,5-42,5 °C	Qualitative	>10 colonies on CCI agar	Yellow color of CSB; small to medium sized (1-3 mm), blue to blue- green colonies on CCI agar
	<i>Cronobacter muytjensii</i> ATCC® 51329 [WDCM 00213] + <i>Staphylococcus aureus</i> ATCC® 25923 [WDCM 00034]				
	<i>Cronobacter dubliensis</i> DSM 18706 + <i>Staphylococcus aureus</i> ATCC® 25923 [WDCM 00034]				
Selectivity	<i>Staphylococcus aureus</i> ATCC® 6538 [WDCM 00032]	22-26 h at 40,5-42,5 °C	Qualitative	Total or partial inhibition, ≤ 100 colonies on Tryptic Soy Agar (TSA)	Purple color of CSB
	<i>Staphylococcus aureus</i> ATCC® 25923 [WDCM 00034]				

Please refer to the actual batch related Certificate of Analysis.

The performance test is in accordance with the current version of EN ISO 11133 and EN ISO 22964.

Literature

ISO International Standardisation Organisation. Microbiology of the food chain - Horizontal method for the detection of *Cronobacter* spp. EN ISO 22964:2017.

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

Iversen C., Lehner A., Mullane N., Marugg J., Fanning S., Stephan R., Joosten H. (2007): The identification of *Cronobacter* spp. (*Enterobacter sakazakii*). J. Clin. Microbiol. **45**: 3814–3816.

Iversen, C. and Druggan, P (2012): Culture media for isolation *Cronobacter* species (*Enterobacter sakazakii*). In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds). pp. 357 – 376. Royal Society of Chemistry, Cambridge, UK.

Stephan R., Grim C.J., Gopinath G.R., Mammel M.K., Sathyamoorthy V., Trach L.H., Chase H.R., Fanning S. and Tall B.D. (2014): Re-examination of the taxonomic status of *Enterobacter helveticus*, *Enterobacter pulveris* and *Enterobacter turicensis* as members of the genus *Cronobacter* and their reclassification in the genera *Franconibacter* gen. nov. and *Siccibacter* gen. nov. as *Franconibacter helveticus* comb. nov., *Franconibacter pulveris* comb. nov. and *Siccibacter turicensis* comb. nov., respectively. Int. J. Syst. Evol. Microbiol. **64**: 3402–3410.

Ordering Information

Product	Cat. No.	Pack size	Other pack sizes available
GranuCult™ CSB (Cronobacter Selective Broth) base acc. ISO 22964	1.20597.0500	500 g	
CSB Selective Supplement (Vancomycin)	1.20595.0010	10 x 1 vial (5 mg each)	
GranuCult™ Buffered Peptone Water acc. ISO 6579, ISO 21528, ISO 22964, FDA-BAM and EP	1.07228.0500	500 g	5 kg, 25 kg
ReadyTube 9 BPW ISO	1.46142.0020	20 x 9 ml	
ReadyTube 1000 BPW ISO	1.46403.0006	6 x 100 ml	
Chromocult® CCI (Chromogenic Cronobacter Isolation) agar acc. ISO 22964	1.20596.0500	500 g	
GranuCult™ Tryptic Soy Agar EP, USP, JP, ISO and FDA-BAM	1.05458.0500	500 g	5 kg
MR-VP (Methyl Red-VOGES-PROSKAUER) Broth	1.05712.0500	500 g	
Phenol-red broth (base)	1.10987.0500	500 g	
Bactident® Oxidase	1.13300.0001	50 strips	

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