Technical Data Sheet

Chromocult® CCI (Chromogenic Cronobacter Isolation) agar acc. ISO 22964

Ordering number: 1.20596.0500

For the selective isolation 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.

Mode of Action

This chromogenic medium contains the chromogenic compound 5-bromo-4-chloro-3-indolyl-α-D-glucopyranoside, a substrate for the detection of α-glucosidase. This enzyme is common in all *Cronobacter*, these appear on the medium as blue to blue-green colored colonies.

This medium contains a basis with moderate nutritivity and selectivity which ensures good growth for *Cronobacter* after pre-enrichment and selective enrichment. It contains sodium desoxycholate to inhibit the growth of gram-positive concomitant flora. Sodium thiosulphate and ammonium(III) citrate are incorporated for the differentiation of *Cronobacter* from hydrogen-sulfide-producing *Enterobacteriaecae*. Sodium chloride maintains the osmotic balance of the medium whilst agar is the solidifying agent.

Typical Composition

Specified by ISO 22964		**Chromocult® CCI (Chromogenic Cronobacter Isolation) agar acc. ISO 22964**	
Tryptic digest of casein	7 g/l	Tryptic digest of casein	7 g/l
Yeast Extract	3 g/l	Yeast Extract	3 g/l
NaCl	5 g/l	NaCl	5 g/l
5-Bromo-4-Chloro-3-Indolyl-ß-D-Glucopyranoside	0.15 g/l	5-Bromo-4-Chloro-3-Indolyl-ß-D-Glucopyranoside	0.15 g/l
Sodium desoxycholate	0.25 g/l	Sodium desoxycholate	0.25 g/l
Ammonium iron(III) citrate	1 g/l	Ammonium iron(III) citrate	1 g/l
Sodium thiosulfate	1 g/l	Sodium thiosulfate	1 g/l
Agar	9-18 g/l	Agar-Agar*	12.1 g/l
Water	1000 ml/l	Water	n/a
pH at 25 °C	7.3 ± 0.2	pH at 25 °C	7.3 ± 0.2

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

Preparation

Dissolve 29.5 g in 1 liter of purified water. Heat in boiling water, and agitate frequently until completely dissolved. Autoclave (15 minutes at 121°C).

After the medium has cooled to 45-50°C pour 18-20 ml into plates.

The prepared medium is clear and yellowish-brown.

There should be no visible moisture on the plates before use. When moisture is present, the plates should be dried for the minimum time required to remove visible moisture, following the procedure as described by EN ISO 11133.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Following the procedure given by EN ISO 22964, inoculate the surface of the medium from the selective enrichment culture CSB (Cronobacter selective broth).

From enrichment culture, mix well and inoculate, by means of a 10 µl loop, the surface of the CCI agar to obtain well-separated colonies.

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

After incubation, examine the chromogenic CCI agar for the presence of typical colonies of presumptive *Cronobacter*.

Typical *Cronobacter* colonies on CCI agar are small to medium-sized (1 mm to 3 mm) and blue to blue-green in colour. Non-*Cronobacter* colonies are often white or white with a green centre, grey or black. Some naturally pigmented colonies of non-*Cronobacter* can appear yellow or red.

This presumptive evidence must be confirmed by carrying out the usual tests, e.g. those described 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 plates can be stored at +2 °C to +8 °C in the dark and protected against evaporation for up to 14 days.

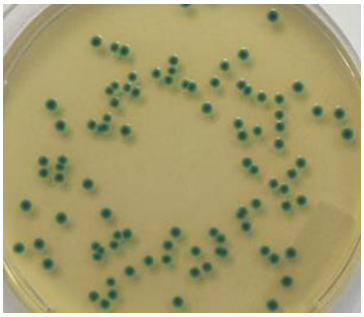
Quality Control

Function	Control strains	Incubation	Reference medium	Method of control	Expected results
Productivity	*Cronobacter sakazakii* ATCC® 29544 [WDCM 00214]	22-26 h at 40,5-42,5°C	Tryptic Soy Agar (TSA)	Quantitative	Recovery ≥70 %, small to medium sized (1-3 mm), blue to blue-green colonies
	Cronobacter muytjensii ATCC® 51329 [WDCM 00213]				
	Cronobacter dubliensis DSM 18706				
	Franconibacter pulveris DSM 19144				
	Siccibacter turicensis DSM 18397				Recovery ≥70 %, small (1-2 mm), light green to light bluegreen colonies
Selectivity	*Staphylococcus aureus* ATCC® 6538 [WDCM 00032]	22-26 h at 40,5-42,5°C	-	Qualitative	Total inhibition
	Staphylococcus aureus ATCC® 25923 [WDCM 00034]				
Specificity	*Enterobacter cloacae* ATCC® 13047 [WDCM 00083]	22-26 h at 40,5-42,5°C	-	Qualitative	Good to very good growth, colonies do not have a green or blue-green color

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.

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



Cronobacter sakazakii WDCM 00124
on Chromocult CCI agar acc. 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
Chromocult® CCI (Chromogenic Cronobacter Isolation) agar acc. ISO 22964	1.20596.0500	500 g	
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	
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™ 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	

Merck KGaA

Frankfurter Strasse 250
64293 Darmstadt, Germany
Fax: +49 (0) 61 51 / 72-60 80

Find contact information for your country at:
www.merckmillipore.com/offices

For Technical Service, please visit:
www.merckmillipore.com/techservice

For more information, visit

www.merckmillipore.com/biomonitoring