

## Technical Data Sheet

### GranuCult® prime

### Colony Count Agar sugar-free acc. ISO 13559 I IDF 153

### Ordering number: 1.10878.0500

For the enumeration of contaminating microorganisms in butter, fermented milks, fresh cheeses and other dairy products.

Colony Count Agar sugar-free acc. ISO 13559 I IDF 153 is also called Plate count sugar-free agar and Sugar-free agar (SFA).

This culture medium complies with the specifications given by ISO 13559 I IDF 153:2002 and the following which refer to this standard: ISO 27205 I IDF 149:2010 and USP Chapter <64>. It also complies with the specification given by APHA.

The performance test of this culture medium complies with the current version of EN ISO 11133.

This culture medium is released by the quality control laboratory of Merck KGaA, Darmstadt, Germany. The laboratory is accredited by the German accreditation authority DAkkS as registered test laboratory D-PL-15185-01-00 according to DIN EN ISO/IEC 17025 for the performance testing of media for microbiology according to DIN EN ISO 11133.

#### Mode of Action

This medium does not contain any fermentable carbohydrates and has relatively low content on nutrients so that contaminating microorganisms can be cultivated selectively. Contaminating microorganisms (non-lactic acid bacteria, yeasts and moulds) are defined as those organisms which are not directly involved in the microbiological production of a dairy product, or which do not belong to its specific flora.

This medium contains enzymatic digests of gelatin and casein which provide a relatively low amount of nutrients. Sodium chloride maintains the osmotic balance and agar is the solidifying agent.

## Typical Composition

ISO 27205 | IDF 149:2010, USP chapter<64> and APHA specify no composition for Colony Count Agar sugar-free.

Specified by ISO 13559   IDF 153:2002		GranuCult® prime Colony Count Agar sugar-free acc. ISO 13559   IDF 153	
Peptone from gelatin	7.5 g/l	Enzymatic digest of gelatin*	7.5 g/l
Peptone from casein	7.5 g/l	Enzymatic digest of casein**	7.5 g/l
Sodium chloride	5.0 g/l	Sodium chloride	5.0 g/l
Agar	10 -15 g/l ***	Agar-Agar ****	15.0 g/l
Water	1000 ml/l	Water	n/a
pH at 25 °C	7.5 ± 0.1	pH at 25 °C	7.5 ± 0.1

\* Enzymatic digest of gelatin is another term for peptone from gelatin, see EN ISO 11133.

\*\* Enzymatic digest of casein is another term for peptone from casein, see EN ISO 11133.

\*\*\* Depending on the gel strength of the agar.

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

## Preparation

Dissolve 35.0 g in 1 liter of purified water. Heat in boiling water and agitate frequently until completely dissolved. Autoclave (15 minutes at 121 °C). Pour to plates.

If the medium is stored in bottles before use, melt and cool it according EN ISO 11133 before pouring plates.

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.

The dehydrated medium is a granulate with beige color.

The prepared medium is clear to slightly opalescent and yellowish.

The pH value at 25 °C is in the range of 7.5 ± 0.1.

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## Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

**Following the procedure given by ISO 13559 I IDF 153**, inoculate plates with the initial suspension of the product and further dilutions by surface plate technique.

Incubate the inoculated plates inverted at  $(30 \pm 1) \text{ }^\circ\text{C}$  for  $(72 \pm 2) \text{ h}$ .

Count the colonies according to the instructions given by ISO 13559 I IDF 153. Do not count pin-point colonies as these are not typical for contaminants.

## Storage

Store at  $+15 \text{ }^\circ\text{C}$  to  $+25 \text{ }^\circ\text{C}$ , 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 ISO 13559 I IDF 153, self-prepared bottled medium can be stored at  $(5 \pm 3) \text{ }^\circ\text{C}$  in the dark for no longer than three months.

## Microbiological Performance

The performance test complies with the current version of EN ISO 11133.

Test method: Performance testing of solid culture media - Quantitative method for solid media (spiral plater)					
Function	Control strains	Incubation	Reference medium	Method of control	Expected results
Productivity	<i>Escherichia coli</i> ATCC® 25922™ [WDCM 00013]	$(72 \pm 2) \text{ h}$ / $(30 \pm 1) \text{ }^\circ\text{C}$ aerobic	Tryptic Soy Agar (TSA)	Quantitative	Recovery $\geq 70 \%$
	<i>Staphylococcus aureus</i> ATCC® 25923™ [WDCM 00034]				
	<i>Enterococcus faecalis</i> ATCC® 11700™ [WDCM -]				
	<i>Pseudomonas aeruginosa</i> ATCC® 27853™ [WDCM 00025]				
	<i>Bacillus cereus</i> ATCC® 11778™ [WDCM 00001]				
	<i>Candida albicans</i> ATCC® 10231™ [WDCM 00054]				

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**Test method: Performance testing of solid culture media - Quantitative method for solid media (streaking method)**

Selectivity / Electivity	<i>Lactiplantibacillus plantarum</i> (formerly <i>Lactobacillus plantarum</i> ) ATCC® 14917™ [WDCM 00104]	(72 ± 2) h/ (30 ± 1) °C aerobic	-	-	no limit pin-point to small colonies
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Please refer to the actual batch related Certificate of Analysis.  
A recovery rate of 70 % is equivalent to a productivity rate of 0.7.

### Literature

APHA (2004) Standard Methods for the Examination of Dairy Products. 17<sup>th</sup> ed. American Public Health Association, Washington, D.C.

EN ISO International Standardisation Organisation. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media + Amendment 1 + Amendment 2. EN ISO 11133:2014/Amd 1:2018/Amd 2:2020.

ISO International Standardisation Organisation. IDF International Dairy Federation. Butter, fermented milks and fresh cheese — Enumeration of contaminating microorganisms — Colony-count technique at 30 °C. ISO 13559:2002 | IDF 153:2002.

ISO International Standardisation Organisation. IDF International Dairy Federation. Fermented milk products — Bacterial starter cultures — Standard of identity. ISO 27205:2010 | IDF 149:2010.

United States Pharmacopeial Convention. (2025): The United States Pharmacopeia (USP). Chapter (64) Probiotic Tests (2024) Rockville, Md., USA.

### Ordering Information

Product	Cat. No.	Pack size
GranuCult® prime Colony Count Agar sugar-free acc. ISO 13559   IDF 153	1108780500	500 g