

## Technical Data Sheet

# GranuCult™

## MRS Agar (de MAN, ROGOSA and SHARPE)

### acc. ISO 15214

Ordering number: 1.10660.0500

For isolation, enumeration and cultivation of *Lactobacillus spp.* and other mesophilic lactic acid bacteria from all types of materials.

This culture medium complies with the specifications given by ISO 15214 and APHA.

#### Mode of Action

This medium contains polysorbate, acetate, magnesium and manganese, which are known to act as special growth factors for *Lactobacilli*, as well as a rich nutrient base. As the medium exhibit a fairly low degree of selectivity, it may support growth of *Pediococcus* and *Leuconostoc* species and other secondary bacteria.

#### Typical Composition

Specified by ISO 15214		GranuCult™ MRS agar (de MAN, ROGOSA and SHARPE) acc. ISO 15214	
Enzymatic Digest of Casein	10 g/l	Enzymatic Digest of Casein	10 g/l
Meat Extract	10 g/l	Meat Extract	10 g/l
Yeast Extract	4 g/l	Yeast Extract	4 g/l
Glucose	20 g/l	D(+)-Glucose	20 g/l
Dipotassium Hydrogen Phosphate	2 g/l	Dipotassium Hydrogen Phosphate	2 g/l
Polyoxyethylenesorbitan monooleate (Tween® 80)	1.08 g/l	Tween® 80	1.08 g/l
Triammonium Citrate	2 g/l	Di-Ammonium Hydrogen Citrate (equivalent to 2 g/l Triammonium Citrate)	2 g/l
Sodium Acetate	5 g/l	Sodium Acetate	5 g/l
Magnesium Sulfate Heptahydrate	0.2 g/l	Magnesium Sulfate Heptahydrate	0.2 g/l
Manganese Sulfate Tetrahydrate	0.05 g/l	Manganese Sulfate Monohydrate (equivalent to 0.05 g/l Manganese sulfate tetrathhydrate)	0.04 g/l
Agar	12-18 g/l	Agar-Agar*	14 g/l
Water	1000 ml/l	Water	n/a
pH at 25 °C	5.7 ± 0.1**	pH at 25 °C	5.6-5.9

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

\*\* ISO 15214 states for the pH: In order that the pH-value does not fall below 5.6, the tolerance here is ± 0.1 instead of ± 0.2 as usual.

## Preparation

Dissolve 68.2 g in 1 l of purified water. Heat in boiling water, and agitate frequently until completely dissolved. Autoclave 15 minutes at 121 °C or use 118 °C to achieve improved growth of *Bifidobacterium* spp.

The prepared medium is clear and brown.

## Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Following the procedure given by ISO 15214, inoculate the medium by using poured plate technique.

Incubate the inoculated plates inverted at 29-31 °C for 69-75 h.

According to ISO 15214, surface plating in combination with incubation under anaerobic or microaerobic conditions can be applied instead of the pour plating method. Anaerobic or microaerobic conditions can be achieved by using Anaerocult® C or C mini in an anaerobic jar.

It is also possible to use a double-layer MRS.

Avoid desiccation of the agar during incubation so that the medium does not become too inhibitory by increasing the acetate concentration at the surface, which inhibits the growth of *Lactobacilli*.

## 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 Corry et al., self-prepared plates can be stored +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	<i>Lactobacillus acidophilus</i> ATCC® 4356	69-75 h at 29-31 °C aerobic	Previously validated batch of MRS agar	Quantitative	Recovery ≥ 70 %
	<i>Lactobacillus sakei</i> ATCC® 15521				
	<i>Lactococcus lactis</i> <i>spp. lactis</i> ATCC® 19435				
	<i>Pediococcus</i> <i>pentosaceus</i> ATCC® 33316				
	<i>Pediococcus</i> <i>pentosaceus</i> ATCC® 29358				
	<i>Bifidobacterium bifidum</i> ATCC® 11863	69-75 h at 29-31 °C anaerobic	-	Qualitative	Good growth



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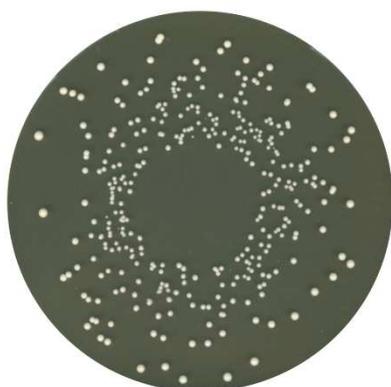
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Function	Control strains	Incubation	Reference medium	Method of control	Expected results
Selectivity	<i>Escherichia coli</i> ATCC® 8739	69-75 h at 29-31 °C	-	Qualitative	Total inhibition
	<i>Escherichia coli</i> ATCC® 25922				
	<i>Bacillus cereus</i> ATCC® 11778				

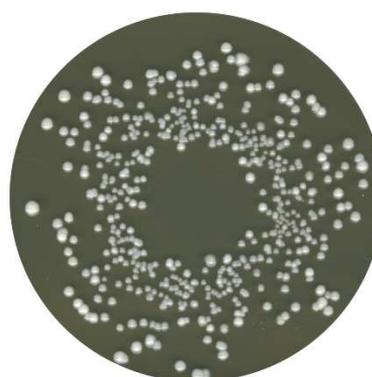
Please refer to the actual batch related Certificate of Analysis.

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

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



*Lactobacillus casei* ATCC® 393



*Bifidobacterium bifidum* ATCC® 11863

## Literature

APHA (2015): Compendium of Methods for the Microbiological Examination of Foods. 5<sup>th</sup> edition. American Public Health Association, Washington, D.C.

De Man J.D., Rogosa M., and Sharpe M.E. (1960): A Medium for the cultivation of *Lactobacilli*. J. Appl. Bact. **23**: 130-135.

ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of mesophilic lactic acid bacteria - Colony-count technique at 30 °C. ISO 15214:1998.

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

Schillinger, U. and Holzapfel, W.H. (2012): Culture media for Lactic Acid Bacteria. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds), pp 174-186. Royal Society of Chemistry, Cambridge, UK.



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## Ordering Information

Product	Cat. No.	Pack size
GranuCult™ MRS Agar (de MAN, ROGOSA and SHARPE) acc. ISO 15214	1.10660.0500	500 g
ReadyPlate™ MRS Medium ISO 15214	1.46717.0020	20 x 90 mm
ReadyPlate™ MRS Medium ISO 15214	1.46717.0100	100 x 90 mm
ReadyTube™ 200 MRS Medium ISO 15214	1.46364.0006	6 x 200 ml
Anaerobic jar	1.16387.0001	1 jar
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® C	1.16275.0001	1 x 10
Anaerocult® C mini	1.13682.0001	1 x 25
Plate basket	1.07040.0001	1 piece

Merck KGaA, 64271 Darmstadt, Germany  
Fax: +49 (0) 61 51 / 72-60 80  
mibio@merckgroup.com  
[www.merckmillipore.com/biomonitoring](http://www.merckmillipore.com/biomonitoring)

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