Technical Data Sheet

GranuCult® prime
TOS-MUP Agar (base) acc. ISO 29981 l IDF 220

Ordering number: 1.00043.0500

For the enumeration of Bifidobacteria from milk products, starter and probiotic cultures and other materials.

TOS-MUP Agar (base) acc. ISO 29981 l IDF 220 is also called TOS Propionate Agar (base), Transgalactosylated Oligosaccharides (TOS)-Mupirocin (MUP) lithium salt Agar (base), and Transgalactosylated Oligosaccharides (TOS)-MUP Agar (base).

This culture medium complies with the specifications given by ISO 29981 I IDF 220:2024. 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 contains a combination of enzymatic digest of casein and yeast extract which gives a rich nutrient base for excellent growth of bifidobacteria. The addition of magnesium sulphate further allows to support growth of pre-injured bifidobacteria. Ammonium sulfate serves as nitrogen source, potassium dihydrogen phosphate and di-potassium hydrogen phosphate buffer the medium in the neutral pH zone. L-Cysteine serves as reducing agent to give the necessary anaerobic condition in the medium. Agar-agar is the gelling agent.

Galactooligosaccharide TOS is a specific growth factor for all bifidobacteria whereas other lactic acid bacteria are not able to utilize this saccharide. Sodium propionate largely inhibits the growth of the accompanying flora.

TOS-MUP agar contains the antibiotic mupirocin lithium salt (MUP), which inhibits the growth of most lactic acid bacteria commonly used in products, such as fermented and non-fermented milks (e.g. pasteurized milks, skim milk, whey protein concentrate), milk powders and infant formulae, as well as

starter and probiotic cultures. Due to the proven selectivity of the antibiotic MUP when added to the base medium, usually there is no growth of typical yoghurt bacteria (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*), mesophilic cultures (e.g. *Lactococcus lactis*), *Lactobacillus acidophilus*, *Lacticaseibacillus casei* and *Lacticaseibacillus rhamnosus* on the medium specified. This property has been tested with a representative number of reference strains and isolates (see IDF Bulletin 411:2007).

The final concentration of MUP in the complete medium is 50 mg/l. The concentration corresponds to the specification given by ISO 29981 l IDF 220:2024.

For the enumeration of bifidobacteria from samples containing only bifidobacteria, TOS agar with or without the antibiotic MUP can be used.

Typical Composition

APHA specifies no composition for TOS-MUP Agar.

Specified by ISO 29981 l IDF 220:2024		**GranuCult® prime TOS-MUP Agar (base) acc. ISO 29981 l IDF 220 and MUP Selective Supplement**	
Enzymatic digest of casein	10.0 g/l	Enzymatic digest of casein	10.0 g/l
Yeast extract	1.0 g/l	Yeast extract	1.0 g/l
KH_2PO_4	3.0 g/l	Potassium dihydrogen phosphate	3.0 g/l
K_2HPO_4	4.8 g/l	di-Potassium hydrogen phosphate	4.8 g/l
$(NH_4)_2SO_4$	3.0 g/l	Ammonium sulfate	3.0 g/l
$MgSO_4 \cdot 7H_2O$	0.2 g/l	Magnesium sulphate heptahydrate	0.2 g/l
(*R*)-cysteine·HCl·H_2O	0.5 g/l	L-cysteine hydrochloride monohydrate	0.5 g/l
Sodium propionate	15.0 g/l	Sodium propionate	15.0 g/l
TOS [Transgalactosylated oligosaccharide (TOS) mixture]	10.0 g/l	Transgalactosylated oligosaccharide (TOS) mixture	10.0 g/l
Agar	9 – 15 g/l*	Agar-Agar**	15.0 g/l
Water	950 ml/l or 1 000 ml/l***	Water	n/a
Supplement to be added after autoclaving:			
Lithium mupirocin (MUP)	50 mg/l	Lithium mupirocin (MUP)	50 mg/l
Water	50 ml/l	Water	n/a
pH at 25 °C	6.7 ± 0.1	pH at 25 °C	6.7 ± 0.1

* Depending on the gel strength of the agar.

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

*** For the preparation of TOS-MUP agar, 950 ml/l water is added and 50 ml /l MUP solution after autoclaving. For the preparation of TOS agar, 1 000 ml/l water is added.

Preparation

For the preparation of TOS-MUP agar, dissolve 62.5 g in 950 ml of purified water. Heat in boiling water and agitate frequently until completely dissolved. The medium is heat sensitive and should be autoclaved (15 minutes at 115 °C) in small portion, 95 or 190 ml. Aseptically add 5 ml of MUP Selective Supplement, Cat No. 100045, to 95 ml of liquefied base medium at 48 °C ± 1 °C (190 ml of base medium is supplemented with 10 supplement solution). The supplement solution is carefully added to the base medium to avoid air bubbles, which can cause oxidation of the medium. Pour to plates.

It is recommended to add the TOS-MUP Selective Supplement (Mupirocin) to the molten TOS-MUP Agar (base) immediately before adding the complete medium to the inoculum using the pour plate technique.

The final concentration of MUP in the complete medium is 50 mg/l. Other volumes can be used when the final concentration is 50 mg/l.

For the preparation of TOS agar, dissolve 62.5 g in 1 liter of purified water. Heat in boiling water and agitate frequently until completely dissolved. The medium is heat sensitive and should be autoclaved (15 minutes at 115 °C) in small portions, 100 ml or 200 ml. Pour to plates.

Alternatively prepare TOS-MUP agar base medium as described in above and add 10 ml of sterile water (instead of MUP solution) to each portion of 190 ml of base medium. Mix well by rotation to minimize foaming. Other volumes may be used provided that the final composition is the same as that specified.

TOS-MUP agar and TOS agar are sensitive to heat, thus excessive heat treatment can negatively influence the properties of the medium. Use the molten medium within 4 h of its preparation.

If the base medium is not used immediately, allow the base medium to solidify in the flask or bottle and store it in the dark at a temperature of 5 °C for no longer than four weeks, under conditions that do not allow any changes in its composition and properties. Before use, melt the base medium completely in a boiling water bath, then cool it in the water bath maintained at 44 °C to 47 °C.

The dehydrated medium is a granulate with beige color.

The prepared medium is clear and yellowish.

The pH value at 25 °C is in the range of 6.7 ± 0.2.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Following the procedure given by ISO 29981 l IDF 220, inoculate plates with the initial suspension of the product and further dilutions by pour plate technique.

Incubate the inoculated plates inverted at (37 ± 1) °C for (72 ± 3) h in an anaerobic atmosphere. Alternatively, 48 h ± 3 h incubation can be applied for the test sample if the colony size is large enough to count accurately.

Count the colonies according to the instructions in by ISO 29981 l IDF 220.

Typical colonies of bifidobacteria are lenticular or round whitish colonies, partially star shaped or trilobate of diameter 1 mm to 4 mm in/on TOS-MUP agar or TOS agar under the conditions specified in ISO 29981 l IDF 220.

Following the procedure given by ISO 29981 l IDF 220, confirmation of presumptive bifidobacteria by microscope observation is required, but optional in the case of test samples containing only bifidobacteria. Optionally, a F6PPK-assay can be performed to confirm the results.

Some strains of bifidobacteria can show differing colony sizes and appearances on the same plate. Most colonies of bifidobacteria give off an acetic acid odor.

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 ISO 29981 l IDF 220, self-prepared TOS base medium (without MUP) can be stored in the flasks or bottles at (5 ± 3) °C in the dark for no longer than four weeks, under conditions that do not allow any changes in its composition and properties.

Microbiological Performance

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

Testing in addition of TOS-MUP Selective Supplement (Cat. No. 1.00045.0010)					
Test method: Quantitative method for solid media (poured plate method)					
Function	**Control strains**	**Incubation**	**Reference medium**	**Method of control**	**Expected results**
Productivity	*Bifidobacterium animalis* subsp. *lactis* ATCC® 27536™ [WDCM -]	(72 ± 4) h/ (37 ± 1) °C anaerobic	Media batch TOS-MUP agar already validated	Quantitative	Recovery ≥ 70 %
	Bifidobacterium breve ATCC® 15700™ [WDCM 00224]				
	Bifidobacterium longum subsp. longum ATCC® 15707™ [WDCM 00225]				

Test method: Performance testing of solid culture media - Quantitative method for solid media (streaking method)					
Selectivity	*Lacticaseibacillus casei* (formerly *Lactobacillus casei*) ATCC® 393™ [WDCM 00100]	(72 ± 2) h/ (30 ± 1) °C aerobic	-	qualitative	Total inhibition
	Lactobacillus delbrueckii subsp. *bulgaricus* ATCC® 11842™ [WDCM 00102]				Total inhibition
	Streptococcus salivarius DSM 20259 [WDCM -]				Total inhibition

Testing without addition of Selective Supplement					
Test method: Quantitative method for solid media (poured plate method)					
Function	**Control strains**	**Incubation**	**Reference medium**	**Method of control**	**Expected results**
Productivity	*Bifidobacterium animalis* subsp. *lactis* ATCC® 27536™ [WDCM -]	(72 ± 4) h/ (37 ± 1) °C anaerobic	Media batch TOS agar already validated	Quantitative	Recovery ≥ 70 %
	Bifidobacterium breve ATCC® 15700™ [WDCM 00224]				
	Bifidobacterium longum subsp. longum ATCC® 15707™ [WDCM 00225]				

Please refer to the actual batch related Certificate of Analysis.
A recovery rate of 70 % is equivalent to a productivity rate of 0.7.

Bifidobacterium longum subsp. *infantis* on GranuCult® prime TOS-MUP Agar (base) acc. ISO 29981 l IDF 220 with TOS-MUP Selective Supplement inoculated by pour plate technique.

Literature

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

APHA (2015) Chapter No. 20: Probiotics. and Chapter No. 67: Microbiological media, reagents and stains. Compendium of Methods for the Microbiological Examination of Foods. 5th 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. Milk products — Enumeration of bifidobacteria — Colony-count technique. ISO 29981:2024 l IDF 220:2024.

International Dairy Federation. Selective enumeration of bifidobacteria in dairy products: Development of a standard method. Bulletin of the IDF n°411, 2007.

International Dairy Federation. Multi-laboratory study and interlaboratory study on the enumeration of bifidobacteria in milk products. Bulletin of the IDF n°530, 2024.

Barrett, E., Mattarelli, P., Simpson, P.J., O'Toole, P.W., Fitzgerald, G.F., Ross, R.P. and Stanton C. (2012): Culture Media for the Detection and Enumeration of Bifidobacteria in Food Production. In: Corry, J.E.L., Curtis, G.D.W., Baird, R.M. (eds) Handbook of Culture media for Food and Water Microbiology. pp. 199-227. Royal Society of Chemistry, Cambridge, UK.

Rada, V. and Koc, J. (2000): The use of mupirocin for selective enumeration of bifidobacteria in fermented milk products. Milchwissenschaft. **55**, pp. 65–67.

Simpson, P.J., Fitzgerald, G.F., Stanton, C. and Ross, R.P. (2004): The evaluation of a mupirocin-based selective medium for the enumeration of bifidobacteria from probiotic animal feed. J. Microbiol. Meth. **57**, pp. 9–16.

Ordering Information

Product	Cat. No.	Pack size
GranuCult® prime TOS-MUP Agar (base) acc. ISO 29981 l IDF 220	1.00043.0500	500 g
MUP Selective Supplement	1.00045.0010	10x 1 vial
Anaerocult® P Reagent for the generation of an anaerobic atmosphere for one Petri dish	1.32382.0001	25 x 1 set
Anaerocult® A mini Gas generator system for the incubation of one to four petri dishes in an anaerobic atmosphere	1.32369.0001	25 x 1 set
Anaerocult® A Reagent for the generation of an anaerobic atmosphere in an anaerobic jar	1.32381.0001	10 x 1 piece
Anaerotest® Test stripes for the detection of an anaerobic atmosphere	1.32371.0001	50 test stripes
Anaerobic jar 2,5 l-volume	1.13681.0001	1 unit

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