

## Technical Data Sheet

### TOS-MUP Selective Supplement (Mupirocin)

Ordering number: 1.00045.0010

TOS-MUP selective supplement contains a lithium mupirocin in lyophilized form.

#### Mode of Action

GranuCult® prime TOS-MUP Agar (base) acc. ISO 29981 I IDF 220 can be used with the addition of TOS-MUP selective supplement which contains lithium mupirocin (MUP) for the enumeration of Bifidobacteria from milk products and other materials.

It utilizes the selective inhibitory properties of 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*, *Lactocaseibacillus casei* and *Lactocaseibacillus 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 I IDF 220:2024.

#### Typical Composition

Ingredient	Gram per vial	Final concentration [g/l]
Lithium mupirocin	0.025	0.050

## Preparation

Add 25 ml of sterile, deionized water to the lyophilizate in the original vial and dissolve by carefully swirling.

Add the solved supplement to the base culture medium, TOS-MUP Agar (base) acc. ISO 29981 I IDF 220, Cat No. 100043, that has been cooled to  $48\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ .

To a preparation with 95 ml of base culture medium add 5 ml of supplement solution. To a preparation with 190 ml of base culture medium add 10 ml of supplement solution. Carefully mix the supplement solution into the base culture medium, avoiding the formation of 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 prepared medium is clear and yellowish.

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.

## Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

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

Incubate the inoculated plates inverted at  $(37 \pm 1)\text{ }^{\circ}\text{C}$  for  $(72 \pm 3)\text{ h}$  in an anaerobic atmosphere. Alternatively,  $48\text{ h} \pm 3\text{ h}$  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 I 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 I IDF 220.

Following the procedure given by ISO 29981 I 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

Usable up to the expiry date when stored dry and tightly closed at +2 to +8 °C. For *in vitro* use only.

## Microbiological Performance

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

TOS-MUP Selective Supplement is tested in GranuCult® prime TOS-MUP Agar (base) acc. ISO 29981 I IDF 220, Cat. No. 1.00043.0500.

Test method: Quantitative method for solid media (poured plate method)					
Function	Control strains	Incubation	Reference medium	Method of control	Expected results
Productivity	<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> ATCC® 27536™ [WDCM -]	(72 ± 4) h/ (37 ± 1) °C anaerobic	Media batch TOS-MUP agar already validated	Quantitative	Recovery ≥ 70 %
	<i>Bifidobacterium breve</i> ATCC® 15700™ [WDCM 00224]				
	<i>Bifidobacterium longum</i> subsp. <i>longum</i> ATCC® 15707™ [WDCM 00225]				
Test method: Performance testing of solid culture media - Quantitative method for solid media (streaking method)					
Selectivity	<i>Lactocaseibacillus casei</i> (formerly <i>Lactobacillus casei</i> ) ATCC® 393™ [WDCM 00100]	(72 ± 2) h/ (30 ± 1) °C aerobic	-	qualitative	Total inhibition
	<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> ATCC® 11842™ [WDCM 00102]				Total inhibition
	<i>Streptococcus salivarius</i> DSM 20259 [WDCM -]				Total inhibition

Please refer to the actual batch related Certificate of Analysis.

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

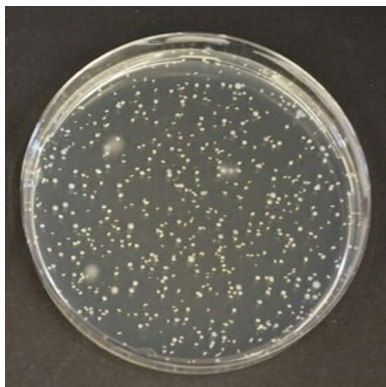
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*Bifidobacterium longum* subsp. *infantis* on GranuCulture® prime TOS-MUP Agar (base)  
acc. ISO 29981 | IDF 220 with TOS-MUP Selective Supplement inoculated by pour plate technique.

## Literature

APHA (2004) Standard Methods for the Examination of Dairy Products. 17<sup>th</sup> 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 | 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.

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## Ordering Information

Product	Cat. No.	Pack size
MUP Selective Supplement	1.00045.0010	10x 1 vial
GranuCult® prime TOS-MUP Agar (base) acc. ISO 29981 I IDF 220	1.00043.0500	500 g
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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