

## Technical Data Sheet

### GranuCult® prime

### OF (Oxidative/Fermentative) medium (base) acc. ISO 21528

Ordering number: 1.03865.0500

For the confirmation and identification of Gram-negative bacteria on the base of oxidative or fermentative metabolism of carbohydrates; with addition of glucose for the confirmation of *Enterobacteriaceae* from products intended for human consumption and the feeding of animals and environmental samples in the area of primary production, food production and food handling.

OF (Oxidative/Fermentative) medium (base) acc. ISO 21528 is also known as HUGH and LEIFSON's OF basal medium (OFBM).

This culture medium complies with the specifications given by EN ISO 21528-1, EN ISO 21528-2, FDA-BAM Medium M117 and APHA.

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 culture medium is used for the determination of the oxidative or fermentative metabolism of a carbohydrate or its nonuse. Bacteria use carbohydrates metabolically by one of two processes, fermentative or oxidative. Some bacteria can metabolize a carbohydrate (as exhibited by acid production) only in aerobic conditions, whilst others produce acid both aerobically and anaerobically. Bacteria that can grow, metabolize and reproduce under either aerobic (presence of atmospheric oxygen) or anaerobic (absence of atmospheric oxygen) conditions are called facultative anaerobes.

A carbohydrate is added to the culture medium, degradation of the carbohydrate to acid is indicated by the pH indicator bromothymol blue which changes its color to yellow. The degradation is allowed to take place while the medium is exposed to air (degradation may be oxidative or fermentative) or under exclusion of air (degradation by fermentation only).

In relation to the peptone concentration this culture medium contains a high concentration of added carbohydrates. This avoids the utilization of peptones by aerobic microorganisms and the resultant alkaline reaction which would neutralize slight acidity produced by an oxidative microorganism. Dipotassium phosphate promotes fermentation and acts as a buffer to control pH. The agar concentration enables the determination of motility, in addition to the oxidative or fermentative ability of a microorganism. Acid produced at the surface of the medium is promoted on its distribution throughout the tube by the agar.

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As in EN ISO 21528-1/-2, OF medium base is mostly used with added glucose (dextrose). By adding other carbohydrates it can be used as well for determination of oxidative-fermentative reaction with lactose, sucrose, maltose, mannitol, xylose and others.

### Typical Composition

Specified by EN ISO 21528-1/-27		Specified by FDA-BAM Medium M117		GranuCult® prime OF (Oxidative/Fermentative) medium (base) acc. ISO 21528	
Enzymatic digest of casein	2.0 g/l	Peptone	2.0 g/l	Enzymatic digest of casein	2.0 g/l
NaCl	5.0 g/l	NaCl	5.0 g/l	NaCl	5.0 g/l
K <sub>2</sub> HPO <sub>4</sub>	0.3 g/l	K <sub>2</sub> HPO <sub>4</sub>	0.3 g/l	K <sub>2</sub> HPO <sub>4</sub>	0.3 g/l
Bromthymol blue	0.08 g/l	Bromthymol blue	0.08 g/l	Bromthymol blue	0.08 g/l
Agar	3 g to 4 g/l*	Agar	2.5 g/l	Agar-agar**	3.5 g/l
<b>Carbohydrate added</b>					
Glucose	10.0 g/l	Glucose (or other)	10.0 g/l	Glucose (or other)	10.0 g/l
Water	1000ml	Water	1 liter	Water	n/a
pH at 25 °C	6.8 ± 0.2	pH at 25 °C	7.1	pH at 25 °C	6.8 ± 0.2

\* Depending on the gel strength of the agar.

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

### Preparation

Dissolve 11.0 g and 10 g of D(+)-Glucose, Cat. No. 108342, in 1 liter of purified water. Heat in boiling water and agitate frequently until completely dissolved.

Dispense in tubes of appropriate capacity (e.g. 10 ml for tubes of 16 mm x 160 mm). Autoclave (15 minutes at 121 °C). Allow to solidify in a vertical position.

#### Addition of other carbohydrates:

Dissolve 11.0 g in 1 liter of purified water. Heat in boiling water and agitate frequently until completely dissolved. Autoclave (15 minutes at 121 °C).

Cool to about 50 °C, add 100 ml/litre of a 10 % filter-sterilized solution of lactose, sucrose, maltose, mannitol, xylose or other carbohydrate, mix.

Dispense in sterile tubes of appropriate capacity (e.g. 10 ml for tubes of 16 mm x 160 mm). Allow to solidify in a vertical position.

If other carbohydrates added than glucose, the final pH of the complete OF medium must be checked and adjusted, if necessary. For adjustment of the pH, follow the instructions given by EN ISO 11133.

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The dehydrated medium is a granulate with beige-greenish color.

The prepared medium is clear to slightly opalescent and blue-green. The pH value at 25 °C is in the range of 6.6 - 7.0 when the added carbohydrate is glucose.

Before inoculation, allow the prepared medium to equilibrate at room temperature if it was stored at a lower temperature.

Just before use for inoculation, heat - at least the tubes intended for the fermentative test - in boiling water or flowing steam for 15 min to remove oxygen, then cool rapidly to the incubation temperature.

### Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Inoculate a pair of OF tubes of each carbohydrate use with each microorganisms being tested.

Using a wire, stab the material from a colony taken from a non-selective medium into two tubes of OF medium with carbohydrate.

The tubes should be stabbed to approximately 0,5 cm (=¼ inch) from the bottom. A light inoculum should be used, the microorganisms used for the inoculum should be in the logarithmic growth phase.

For each test strain, overlay the surface of one of the tubes with minimal 1 cm of sterile mineral oil or paraffin viscous, Cat. No. 107160.

Incubate at least 48 h at (35 ± 2) °C with loosen caps. Do not discard as negative until after 4 days of incubation.

A yellow coloration in both, the open and paraffin sealed tubes, signifies fermentative degradation whereas yellow coloration of the open tubes alone indicate that the carbohydrate in question is broken down by oxidation. Oxidative breakdown takes place at or close to the surface of the medium, whilst fermentative breakdown occur both at the surface and throughout the butt. If the carbohydrate is not utilized by either method, there is no acid production (= no yellow coloration) in either tube.

The tubes should finally be checked to see whether microbial growth produces turbidity solely along the inoculation line (immotile strain) or throughout the whole medium (motile strain).

#### Confirmation test according EN ISO 21528-1/-2:

Only those colonies need to be tested on glucose fermentation which gave a negative Oxidase test. These colonies are tested by stabbing material from the same colony as used for the Oxidase test into tubes containing OF Medium with Glucose. Overlay the surface of this tube with minimal 1 cm of sterile mineral oil or paraffin viscous, Cat. No. 107160.

According EN ISO 21528-1/-2, incubate at (37 ± 1) °C for (24 ± 2) h aerobically.

If a yellow color develops throughout the content of the tube, regard the reaction as being positive for glucose fermentation test.

### 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.

Acc. to ISO 21528-1/-2, self-prepared tubes can be stored at (5 ± 3 °C) for up to 4 weeks in the dark and protected against evaporation.

Just before use for inoculation, heat the tubes, see Preparation.

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## Microbiological Performance

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

Test method: Performance testing of solid culture media - Qualitative method for confirmation media and reagents.

Test strain	Specification	
	Oxidative (Tube without overlay)	Fermentative (Tube with overlay)
<i>Escherichia coli</i> ATCC® 8739 [WDCM 00012]	good growth, yellow color in the whole tube	good growth, yellow color in the whole tube
<i>Escherichia coli</i> ATCC® 25922 [WDCM 00013]	good growth, yellow color in the whole tube	good growth, yellow color in the whole tube
<i>Escherichia coli</i> ATCC® 11775 [WDCM 00090]	good growth, yellow color in the whole tube	good growth, yellow color in the whole tube
<i>Escherichia coli</i> DSM 19683 [WDCM 00179]	good growth, yellow color in the whole tube	good growth, yellow color in the whole tube
<i>Pseudomonas aeruginosa</i> ATCC® 10145 [WDCM 00024]	growth, yellow color only at the top of the tube, rest of the medium remains blue- green	growth, no yellow color, medium remains blue-green
<i>Pseudomonas aeruginosa</i> ATCC® 27853 [WDCM 00025]	growth, yellow color only at the top of the tube, rest of the medium remains blue- green	growth, no yellow color, medium remains blue-green
<i>Pseudomonas aeruginosa</i> ATCC® 9027 [WDCM 00026]	growth, yellow color only at the top of the tube, rest of the medium remains blue- green	growth, no yellow color, medium remains blue-green
<i>Pseudomonas fluorescens</i> ATCC® 13525 [WDCM 00115]	growth, no yellow color, medium remains blue-green	growth, no yellow color, medium remains blue- green

Incubation: 24 ± 2 h at 37 ± 1 °C,  
with and without an overlay of minimal 1 cm sterile mineral oil on the surface of the medium.

Please refer to the actual batch related Certificate of Analysis.

## Literature

APHA (2015) Chapter No. 67: Microbiological media, reagents and stains. Compendium of Methods for the Microbiological Examination of Foods. 5<sup>th</sup> ed. American Public Health Association, Washington, D.C.

EN ISO International Standardisation Organisation. Microbiology of the food chain – Microbiology of the food chain – Horizontal method for the detection and enumeration of Enterobacteriaceae – Part 1: Detection of *Enterobacteriaceae*. EN ISO 21528-1:2017.

EN ISO International Standardisation Organisation. Microbiology of the food chain – Microbiology of the food chain – Horizontal method for the detection and enumeration of *Enterobacteriaceae* – Part 2: Colony-count technique. EN ISO 21528-2:2017.

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/Amd1:2018/Amd2:2020.

FDA-BAM (2017): Chapter No. 23: Methods for cosmetics. U.S. Food and Drug Administration - Bacteriological Analytical Manual.

FDA-BAM (2018): Media Index for BAM - BAM Media M117: Oxidative-Fermentative (OF) Test Medium. Food and Drug Administration - Bacteriological Analytical Manual.

Hugh, R., and Leifson, E. (1953): The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria. *J. Bact.*, **66**: 24-26.

Druggan, P. and Iversen, C. (2012): Culture media for food spoilage bacteria of the order *Pseudomonadales*: *Pseudomonas*, *Acinetobacter* and *Psychrobacter* spp. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds). pp. 482-507. Royal Society of Chemistry, Cambridge, UK.

MacFaddin, J.F. (2000): Biochemical test for identification of medical bacteria. pp. 379-387. 3<sup>rd</sup> ed. Lippincott, Williams & Wilkins, Baltimore, MD, USA.

MacFaddin, J.F. (1985): Media for isolation – cultivation – identification – maintenance of medical bacteria. Vol 1. 591-597. Williams & Wilkins, Baltimore, MD, USA.

## Ordering Information

Product	Cat. No.	Pack size	Other pack sizes available
GranuCult® prime OF (Oxidative/Fermentative) medium (base) acc. ISO 21528	1.00865.0500	500 g	
GranuCult® Buffered Peptone Water acc. ISO 6579, ISO 19250, ISO 21528, ISO 22964, ISO 6887, FDA- BAM and EP	1.07228.0500	500 g	5 kg, 25 kg
GranuCult® VRBD (Violet Red Bile Dextrose) agar acc. EP, USP, JP and ISO 21528	1.10275.0500	500 g	5 kg
GranuCult® Nutrient agar acc. ISO 6579, ISO 10273 and ISO 21528	1.05450.0500	500 g	
GranuCult® Tryptic Soy Agar EP, USP, JP, ISO, FDA-BAM	1.05458.0500	500 g	5 kg
GranuCult® Peptone salt solution (Maximum recovery diluent) acc. ISO 6887 and ISO 8199	1.12535.0500	500 g	
Ringer tablets	1.15525.0001	100 tablets	
Bactident® Oxidase	1.00181.0001	50 test strips	

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