

Technical Data Sheet

GranuCult™

MMGA (Mineral Modified Glutamate) Agar

acc. ISO 16649

Ordering number: 1.09045.0500

For the resuscitation before enumeration of cells of *Escherichia coli* that might have been subjected to stress from food and feeding stuff as well as from environmental samples in the area of food production and food handling.

This culture medium complies with the specifications given by ISO 16649-1 and those given by ISO 11866-2 I IDF 170-2.

The colony-count technique using membranes acc. to ISO 16649-1 and ISO 11866-2 I IDF 170-2 is suitable for the enumeration of cells of *E. coli* that might have been subjected to stress, e.g. arising from dehydration, freezing, freeze-drying, acidification or damage by disinfectants such as chlorine-containing products.

Mode of Action

The combination of glutamate with selected minerals results in very rapid and effective resuscitation of damaged *E. coli*. After incubation for 4 hours at 37 °C, the bacteria repair process is advanced that growth on a selective culture medium (e.g. Chromocult® TBX Agar acc. ISO 16649) at 44 °C is enabled.

Typical Composition

Specified by ISO 16649-1 and ISO 11866-2 I IDF 170-2		GranuCult™ MMGA (Mineral Modified Glutamate) Agar acc. ISO 16649	
Sodium Glutamate	6.35 g/l	Sodium Glutamate	6.35 g/l
Lactose	10 g/l	Lactose	10 g/l
Sodium Formate	0.25 g/l	Sodium Formate	0.25 g/l
L(-)-Cystine	0.02 g/l	L-Cystine	0.02 g/l
L(-)-Aspartic Acid	0.02 g/l	L-Aspartic Acid	0.02 g/l
L(+)-Arginine	0.024 g/l	L-Arginine Monohydrochloride*	0.029 g/l
Thiamine	0.001 g/l	Thiamine Dichloride	0.001 g/l
Nicotinic Acid	0.001 g/l	Nicotinic Acid	0.001 g/l
Pantothenic Acid	0.001 g/l	Calcium D Pantothenate**	0.001 g/l
Magnesium Sulfate Heptahydrate	0.1 g/l	Magnesium Sulfate Heptahydrate	0.1 g/l
Ammonium Iron(III) Citrate	0.01 g/l	Ammonium Iron(III) Citrate	0.01 g/l
Calcium Chloride Dihydrate	0.01 g/l	Calcium Chloride Dihydrate	0.01 g/l
Dipotassium Hydrogen Phosphate	0.9 g/l	Dipotassium Hydrogen Phosphate	0.9 g/l
Ammonium Chloride	2.5 g/l	Ammonium Chloride	2.5 g/l
Agar	9-18 g/l	Agar-Agar ***	10 g/l
Water	1000 ml/l	Water	n/a
pH at 25 °C	6.7 ± 0.2	pH at 25 °C	6.7 ± 0.2

* L-arginine monohydrochloride 0.029 g/l is equivalent to L(+)-Arginine 0.024 g/l.

** Calcium D pantothenate 0.001 g/l is equivalent to Pantothenic acid 0.001 g/l.

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

Preparation

Dissolve 30.2 g in 1 l of purified water. Heat in boiling water and agitate frequently until completely dissolved. Autoclave 10 minutes at 115 °C and pour plates.

The prepared culture medium is yellowish and clear.

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 agar should be dry enough to allow excess moisture to disappear within 15 min of spreading the inoculum of 1 ml.

Experimental Procedure and Evaluation

Colony-count technique using membranes and TBX agar acc. to ISO 16649-1:

The method is suitable for the enumeration of cells of *E. coli* that might have been subjected to stress.

Using sterile forceps, aseptically place a sterile cellulose membrane with a pore size of 0.45 µm and a diameter of 85 mm onto the dried surface of each of two plates Mineral-modified glutamate agar (MMGA) (article number 1.09045.0500), taking care to avoid trapping air bubbles beneath the membranes. Gently flatten the membranes with a sterile spreader, if necessary.

Using a sterile pipette, add 1 ml of the test sample or the initial suspension to the center of each membrane. Using a sterile spreader, spread the inoculum evenly over the whole membrane surface, avoiding any spillage from the membrane.

Repeat the procedure with the further decimal dilutions. If necessary, using another sterile pipette and another sterile spreader for each dilution.

Leave the inoculated plates in a horizontal position at room temperature for approximately 15 min until the inoculum has soaked through the membrane into the agar.

Incubate the plates at 36-38 °C for 3-5 h, with the membrane/agar surface uppermost.

After resuscitation, transfer membranes using sterile forceps from MMGA (resuscitation medium) to plates of Chromocult® TBX (Tryptone Bile X-glucuronide) agar acc. ISO 16649 (article number 1.16122.0500).

Incubate the TBX plates at 43-45 °C for 20-24 h, with the membrane/agar surface uppermost. Do not stack dishes more than three high.

Examine the TBX agar for the presence of blue or blue green colonies, indicating the presence of β -glucuronidase positive *E. coli* and count the typical CFU (colony-forming units).

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 16649-1, self-prepared plates can be stored at +2 °C to +8 °C in the dark and protected against evaporation for up to 5 days.

Quality Control

Function	Control strains	Incubation	Method of control	Expected results
Productivity	<i>Escherichia coli</i> ATCC® 25922	3-5 h at 36-38 °C on MMGA Agar, afterwards 18-24 h at 43-45 °C on TBX Agar	Quantative	Recovery > 70 %, turquoise colonies blue-green colonies on Chromocult® TBX agar acc. ISO 16649
	<i>Escherichia coli</i> ATCC® 11775			
Selectivity	<i>Citrobacter freundii</i> ATCC® 8090	3-5 h at 36-38 °C on MMGA Agar, afterwards 18-24 h at 43-45 °C on TBX Agar	Qualitative with membrane	Recovery < 0.01 %
	<i>Enterococcus faecalis</i> ATCC® 19433			

Please refer to the actual batch related Certificate of Analysis.

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

Literature

Holbrook R., Anderson J. M. and Baird-Parker A. C. (1980): Modified direct plate method for counting *Escherichia coli* in foods. Food Technology (Australia). **32**: 78-83.

ISO International Standardisation Organisation. Milk and milk products - Enumeration of presumptive *Escherichia coli* - Part 2: Colony-count technique at 44 °C using membranes. ISO 11866-2 I IDF 170-2:2005.

ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* - Part 1: Colony-count technique at 44 °C using membranes and 5-bromo-4-chloro-3-indolyl beta-D-glucuronide. ISO 16649-1:2001.

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

Ordering Information

Product	Cat. No.	Pack size
GranuCult™ MMGA (Mineral Modified Glutamate) Agar acc. ISO 16649	1.09045.0500	500 g
Chromocult® TBX (Tryptone Bile X-glucuronide) Agar acc. ISO 16649	1.16122.0500	500 g
ReadyPlate™ CHROM TBX Agar ISO 16649	1.46326.0020	20 x 90 mm
ReadyPlate™ CHROM TBX Agar ISO 16649	1.46326.0100	100 x 90 mm

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Find contact information for your
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www.EMDmillipore.com/offices

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www.EMDmillipore.com/techservice

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