Technical Data Sheet

GranuCult™ MKTTn (MULLER-KAUFFMANN Tetrathionate Novobiocin) Broth (Base) acc. ISO 6579

Ordering number: 1.05878.0500

For the selective enrichment of *Salmonella* from food, animal feed and other materials.

This culture medium complies with the specifications given by EN ISO 6579 and EN ISO/ FDIS 6579-1.

Mode of Action

Tetrathionate is produced from thiosulfate by adding iodine to the culture medium. Tetrathionate suppresses the growth of coliform and other enteric bacteria. *Salmonella*, *Proteus* and some other species of bacteria can reduce tetrathionate and are not inhibited. Calcium carbonate buffers the sulphuric acid, which is generated when tetrathionate is reduced. Bile promotes the growth of *Salmonella*, but largely inhibits the accompanying bacteria. Brilliant green and novobiocin suppress the growth of primarily Gram-positive bacteria.

For the detection of some *Salmonella* serovars, other culture steps, e.g. other selective enrichment media, may be needed. For *Salmonella* Typhi and *Salmonella* Paratyphi, the procedure is described by EN ISO/FDIS 6579-1.

Typical Composition

Specified by ISO 6579		GranuCult™ MKTTn (MULLER-KAUFFMANN Tetrathionate Novobiocin) Broth (Base) acc. ISO 6579	
Meat Extract	4.3 g/l	Meat Extract	4.3 g/l
Enzymatic Digest of Casein	8.6 g/l	Enzymatic Digest of Casein	8.6 g/l
NaCl	2.6 g/l	NaCl	2.6 g/l
$CaCO_3$	38.7 g/l	$CaCO_3$	38.7 g/l
Sodium Thiosulfate x 5 H_2O	47.8 g/l	Sodium Thiosulfate, anhydrous	30.5 g/l *
Ox Bile for bacteriological use	4.78 g/l	Ox bile	4.78 g/l
Brilliant Green	0.0096 g/l	Brilliant Green	0.0096 g/l
Novobiocin Sodium Salt	0.04 g/l	Novobiocin Sodium Salt	0.04 g/l
Water	1000 ml/l	Water	n/a

Regent to be added immediately before use:			
Potassium Iodide	5 g/l	Potassium Iodide	5 g/l
Iodine	4 g/l	Iodine	4 g/l
Water	20 ml/	Water	20 ml/
pH at 25 °C	8.0 ± 0.2	pH at 25 °C	8.0 ± 0.2

* equivalent to 47.8 g/l sodium thiosulfate x 5 H_2O

Preparation

Dissolve 89.5 g in 1 l of purified water, heat briefly to boiling. **Do not autoclave!** After cooling, add 20 ml/l Iodine-potassium iodide solution. Dispense evenly any precipitate.

Prior to use add 20 ml iodine/potassium iodide solution (see below) to 1000 ml of basal medium.

Dispense the medium aseptically into containers of suitable capacity to obtain the portions of the test, e.g. 10 ml quantities into tubes. Avoid further heating.

The prepared medium is turbid and green with a white sediment (calcium carbonate). The pH value at 25 °C is in the range of 7.8-8.2.

According to EN ISO/FDIS 6579-1, after preparation the pH of complete MKTTn broth will be approximately 8.0. If the complete medium is not used immediately, store in the dark at +2 °C to +8 °C. The pH may drop during storage due to chemical reactions. Do not use the complete medium if the pH drops below 7.0.

Preparation the Iodine/Iodide solution: Completely dissolve 5 g Potassium Iodide (article number 1.05043.0250) in 2 ml of water, then add 4 g Iodine (article number 1.04761. 0100) and dilute to 20 ml distilled water.

According to EN ISO/FDIS 6579-1, the prepared Iodine/Iodide solution can be stored in a (tightly) closed container in the dark for up to one year.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Allow the MKTTn broth to equilibrate at room temperature if it was stored at a lower temperature.

According to EN ISO 6579, transfer 1 ml of the culture obtained in the pre-enrichment (Buffered Peptone Water) to a tube containing 10 ml of MKTTn broth. Minimize the transfer of particulate material from the pre-enrichment into the selective enrichment medium.

Incubate the inoculated broth under aerobic conditions, e.g. according to EN ISO 6579 36-38 °C for 21-27 h.

From the culture obtained in MKTTn broth selective solid media are inoculated, see details given by EN ISO 6579 or other appropriate standard.

According to EN ISO/FDIS 6579-1, for some products it may be necessary to incubate the selective enrichment medium for an additional 24 h, then follow the same plating-out procedure as described above.

According to EN ISO/FDIS 6579-1, it is permissible to store the selective enrichment after incubation at +2 °C to +8 °C for a maximum of 72 h.

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 EN ISO/FDIS 6579-1, self-prepared base medium can be stored in closed containers at +2 °C to +8 °C in the dark and protected against evaporation for up to four weeks. For storage of the self-prepared complete medium, see remarks above under “Preparation”.

Quality Control

Function	Control strains	Incubation	Method of control	Criteria	Expected results
Productivity	*Salmonella* Enteritidis ATCC® 13076 + *Escherichia coli* ATCC® 8739 + *Pseudomonas aeruginosa* ATCC® 27853	21-27 h at 36-38 °C	Qualitative	> 10 colonies on XLD	Colonies with black center on XLD agar acc. ISO 6579
	Salmonella Typhimurium ATCC® 14028 + *Escherichia coli* ATCC® 25922 + *Pseudomonas aeruginosa* ATCC® 27853				
Selectivity	*Escherichia coli* ATCC® 8739	21-27 h at 36-38 °C	Qualitative	Partial inhibition ≤ 100 colonies on Tryptic Soy Agar (TSA)	-
	Escherichia coli ATCC® 25922				
	Enterococcus faecalis ATCC® 19433			< 10 colonies on Tryptic Soy Agar (TSA)	
	Enterococcus faecalis ATCC® 29212				

Please refer to the actual batch related Certificate of Analysis.

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

Literature

Becker, H., Eberhardt, S. and Märtlbauer, E. (2003): Comparative studies on the detection of *Salmonellae* in milk and milk products using a horizontal (ISO 6579:2002) and a vertical (ISO 6785/IDF 93:2001) International Standard. Arch. Lebensmittelhyg. **54**: 118-121.

Edel, W., and Kampelmacher, E.H. (1969): *Salmonella* isolation in nine European laboratories using a standardized technique. Bull. Wld. Hlth. Org. **41**: 297-306.

ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp. EN ISO 6579:2002.

ISO International Standardisation Organisation. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Horizontal method for the detection of *Salmonella* spp. EN ISO/FDIS 6579-1:2015.

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

Mooijman, K.A. (2012): Culture media for the isolation of *Salmonella*. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds). pp. 261-286. Royal Society of Chemistry, Cambridge, UK.

Ordering Information

Product	Cat. No.	Pack size	Other pack sizes available
GranuCult™ MKTTn (MULLER-KAUFFMANN Tetrathionate Novobiocin) Broth (Base) acc. ISO 6579	1.05878.0500	500 g	
Potassium Iodide	1.05043.0250	250 g	
Iodine resublimed	1.04761.0100	100 g	
Tetrathionate Broth (MKTTn)	1.46221.0020	20 x 10 ml	100 x 10 ml
GranuCult™ Buffered Peptone Water acc. ISO 6579, ISO 21528, ISO 22964, FDA-BAM and EP	1.07228.0500	500 g	5 kg, 25 kg
Readybag® Buffered Peptone Water acc. ISO 6579, ISO 21528, ISO 22964, FDA-BAM and EP, 5,7 g, irradiated	1.02448.0060	60 bags	60 bags x 29g 35 bags x 86g
ReadyTube™ 9 BPW ISO 6579, 6887, 21528	1.46142.0020	20 x 9 ml	100 x 9 ml, 6 x 225 ml, 6 x 1000 ml, 1 x 2000 ml
GranuCult™ RVS (RAPPAPORT-VASSILIADIS -Soya) Broth (Base) acc. ISO 6579	1.07700.0500	500 g	
Novobiocin Sodium Salt	N6160-1-G	1 g	5 g, 25 g
ReadyTube™10 RVS Broth ISO 6579	1.46694.0020	20 x 10 ml	100 x 10 ml
MSRV (Modified Semi-Solid RAPPAPORT-VASSILIADIS) Medium (Base) acc. ISO 6579	1.09878.0500	500 g	
MSRV Selective Supplement	1.09874.0010	10 x 1 vial	

Product	Cat. No.	Pack size	Other pack sizes available
ReadyTube™12 MSRV Medium ISO 6579	1.46694.0100	100 x 12 ml	
GranuCult™ XLD (Xylose Lysine Deoxycholate) Agar acc. ISO 6579	1.05287.0500	500 g	
ReadyPlate™ XLD Agar ISO 6579	1.46751.0020	20 x 90 mm	
RAMBACH® Agar	1.07500.0001	4 x 250 ml	4 x 1000 ml 4 x 50 l
RAMBACH® Agar ready-to-use	1.46719.0020	20 x 90 mm	100 x 90 mm
Singlepath® Salmonella	1.04140.0001	25 x 1 test	
Bismuth Sulfite Agar acc WILSON-BLAIR	1.05418.0500	500 g	
Triple Sugar Iron Agar	1.03915.0500	500 g	
Urea Agar (Base) acc. CHRISTIANSEN	1.08492.0500	500 g	
Urea GR for Analysis ACS, Reagent Ph Eur	1.08487.0500	500 g	
MR-VP (Methyl Red-VOGES-PROSKAUER) Broth	1.05712.0500	500 g	
KOVACS' Indole Reagent	1.09293.0100	100 ml	

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