Technical Data Sheet

GranuCult® prime
SIM (Sulfite Indole Motility) Agar
acc. ISO 15213

Ordering number: 1.05470.0500

For the confirmation of *Clostridium perfringens* from samples belonging to the food chain and the differentiation/identification of bacteria from the order *Enterobacterales* from various materials, based on motility as well as sulfite and indole production.

SIM (Sulfite Indole Motility) Agar acc. ISO 15213 is also known as SIM Motility Medium and Sulfide indole motility (SIM) test medium.
This culture medium complies with the specifications given by EN ISO 15213-2:2023, EN ISO/TS 15213-3:2024, APHA, GB 4789.30:2016.

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 semi-solid culture medium allows the differentiation of bacteria by ability to produce hydrogen sulfite (H_2S), indole and to exhibit motility. These distinguishing characteristics aid in the differentiation of bacteria from the order Enterobacterales and also for the confirmation of *Clostridium perfringens*.

The culture medium contains ferrous ammonium sulfate and sodium thiosulfate, which together serve as indicators for hydrogen sulfide production. The ferrous ammonium sulfate reacts with H_2S gas to produce ferrous sulfide precipitates that blackens the medium.

Due to the semi-solid nature of the medium, motile organisms are to swarm from the central inoculation stab line and to produce turbidity or cloudiness throughout the medium. Non-motile bacteria grow only along the puncture line and leave the surrounding medium clear.

The culture medium contains peptone, which contains an enzymatic digest of casein with a high tryptophan content. Certain bacteria are able to produce indole from tryptophan, which can be detected by the addition of Kovacs reagent. A red coloured ring on the top of the medium immediately after the addition of Kovacs reagent indicates a positive reaction, while a negative reaction shows no colour change.

Typical Composition

Specified by EN ISO 15213-2:2023, EN ISO/TS 15213-3:2024		Specified by GB 4789.30:2016		GranuCult® prime SIM (Sulfite Indole Motility) Agar acc. ISO 15213	
Peptone*	20 g/l	Tryptone*	20 g/l	Peptone including enzymatic digest of casein	20 g/l
Enzymatic digest of soya	6 g/l	Poly peptone	6 g/l	Ezymatic digest of soya	6 g/l
Ferrous ammonium sulfate (anhydrous)	0.2 g/l	Ammonium ferric sulfate	0.2 g/l	Ferrous ammonium sulfate, anhydrous	0.2 g/l
Sodium thiosulfate	0.2 g/l	Sodium thiosulfate	0.2 g/l	Sodium thiosulfate	0.2 g/l
Agar	2.5-4.5 g/l**	Agar	3.5 g/l	Agar-Agar***	3.6 g/l
Water	1000 ml/l	Water	1000 ml/l	Water	n/a
pH at 25 °C	7.3 ± 0.2	pH at 25 °C (before autoclaving)	7.2 ± 0.2	pH at 25 °C	7.3 ± 0.2

* For example, enzymatic digest of casein. Tryptone is equivalent to enzymatic digest of casein.
** Depending on the gel strength of the agar.
*** Agar-Agar is equivalent to other different terms of agar.

Preparation

Dissolve 30.0 g in 1 liter of purified water. Heat in boiling water and agitate frequently until completely dissolved. Dispense 10 ml or adequate volume into tubes to give a depth of about 4 cm. Autoclave (15 minutes at 121°C). Allow to solidify in a vertical position.

The dehydrated medium is a granulate with beige color.

The prepared medium is clear and yellowish-brown. The pH value at 25 °C is in the range of 7.3 ± 0.2.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

For the confirmation of *Clostridium perfringens*, follow the procedure given by EN ISO 15213-2:2023 or EN ISO/TS 15213-3:2024. Colonies grown anaerobically on Columbia blood agar or another nutrient-rich medium (e.g. Tryptone soya agar or Brain heart infusion agar) are stabbed into SIM agar tubes.

The tubes are incubated for (22 ± 2) h at (37 ± 1) °C in an anaerobic atmosphere with loosen caps.

After incubation the tubes are read for:

— sulfite production: tubes showing blackening are positive;

— motility: tubes showing growth outside the inoculation stab are positive;

— indole production: tubes giving a red coloured ring directly after adding Kovacs indole reagent are positive.

Clostridium perfringens is positive for sulfite production and negative for indole production and motility.

Diffuse, unspecific blackening of the medium can occur. The growth of anaerobic bacteria, which produce hydrogen (not H_2S), can also reduce the sulfite present and lead to a general blackening of the medium, which makes it difficult to read the typical reactions.

For the differentiation/identification of bacteria from the order *Enterobacterales*, material from a well isolated colony of a pure culture is stabbed into SIM agar tubes.

Incubate the tubes (22 ± 2) h at 37 ± 1 °C, aerobic with loosen caps.

Motility is indicated by a diffuse turbidity of the culture medium surrounding the puncture line. In case of immotility, growth takes place solely along the puncture line. H_2S formation is shown by a blackening in those areas of the medium in which microbial growth has occurred.
After checking the tubes for motility and H_2S production, the indole test is performed. The medium is covered with a layer of Kovacs indole reagent. Production of indole causes the reagent layer to become red in colour.

Non-motile mucoid *Klebsiella* strains may give a false positive motility reaction; this is due to mucoid strains spilling between medium and the tube giving a cloudy appearance which is often confused with motility. False positives can be avoided by use of media with adequate tube depth and careful reading with attention to the density of growth in the central stab.

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 MacFaddin (1985), self-prepared medium can be stored in closed tubes at (5 ± 3) °C for up to 8 weeks in the dark.

Microbiological Performance

The performance test is in accordance with the current versions of EN ISO 11133, EN ISO 15213-2 and EN ISO/TS 15213-3.

Test method: Qualitative method for confirmation media and reagents			
Control strains	**Incubation**	**Expected results**	**Specified by**
Clostridium perfringens ATCC® 13124™ [WDCM 00007]	(22 ± 2) h / (37 ± 1) °C with loosen caps, anaerobic atmosphere	- Good to very good growth - Blackening (sulfite production) of the tube - No growth outside the inoculation stab (motility) - No red coloured ring after adding Kovacs reagent (indole)	EN ISO 15213-2:2023 and EN ISO/TS 15213-3:2024
Clostridium perfringens ATCC® 12916™ [WDCM 00080]			
Clostridium perfringens ATCC® 10543™ [WDCM 00174]			
Escherichia coli ATCC® 8739™ [WDCM 00012]		- Good to very good growth - No blackening (sulfite production) of the tube - Possible growth outside the inoculation stab motility) - Red coloured ring after adding Kovacs reagent (indole)	
Escherichia coli ATCC® 25922™ [WDCM 00013]			
Escherichia coli ATCC® 25922™ [WDCM 00013]	Incubation: (22 ± 2) h / (37 ± 1) °C with loosen caps, aerobic	- Good to very good growth - No blackening (sulfite production) of the tube - Possible growth outside the inoculation stab (motility) - Red coloured ring after adding Kovacs reagent (indole)	
Enterobacter cloacae ATCC® 13047™ [WDCM 00083]		- Good to very good growth - No blackening (sulfite production) of the tube - Growth outside the inoculation stab (motility) - No red coloured ring after adding Kovacs reagent (indole)	
Samonella Typhimurium ATCC® 14028™ [WDCM 00031]		- Good to very good growth - Blackening (sulfite production) of the tube - Growth outside the inoculation stab (motility) - No red coloured ring after adding Kovacs reagent (indole)	
Klebsiella pneumoniae ATCC® 13883™ [WDCM 00097]		- Good to very good growth - No blackening (sulfite production) of the tube - No growth outside the inoculation stab (motility) - No red coloured ring after adding Kovacs reagent (indole)	
Proteus hauseri ATCC® 13315™ [WDCM -]		- Good to very good growth - Blackening (sulfite production) of the tube - Growth outside the inoculation stab (motility) - Red coloured ring after adding Kovacs reagent (indole)	

Please refer to the actual batch related Certificate of Analysis.

Literature

EN ISO International Standardisation Organisation. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Clostridium* spp. — Part 2: Enumeration of *Clostridium perfringens* by colony-count technique. ISO 15213-2:2023.

EN ISO International Standardisation Organisation. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Clostridium* spp. — Part 3: Detection of *Clostridium perfringens*. EN ISO/TS 15213-3:2024.

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.

Blazevic, D.J. (1968): Improved Motility-Indole Medium. Appl. Microbiol. **16**: 668.

Edmondson, E.B., and Sanford, J.P. (1967): The *Klebsiella-Enterobacter (Arcobacter)-Serratia* group. Medicine **46(4)**, 323.

Fischer, M., Zhu, S. and de Ree, E. (2012): Culture media for the detection and enumeration of *Clostridia* in food. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds). pp. 66-89. Royal Society of Chemistry, Cambridge, UK.

MacFaddin, J.F. (1985): Media for isolation – cultivation – identification – maintenance of medical bacteria. Vol 1. SIM (Sulfide Indole Motility) Medium. pp. 711 – 713. Williams & Wilkins, Baltimore, MD, USA.

MERCK

Ordering Information

Product	Cat. No.	Pack size
GranuCult® prime SIM (Sulfite Indole Motility) Agar acc. ISO 15213	1.05470.0500	500 g
Bactident® Indole (KOVÁCS Indole reagent) acc. ISO and FDA-BAM	1.11350.0001	1 x 30 ml
KOVÁCS Indole reagent acc. ISO and FDA-BAM	1.09293.0100	100 ml
GranuCult® prime TSC Agar (Tryptose Sulfite Cycloserine) Agar (base) acc. ISO 15213 and ISO 14189	1.11972.0500	500 g
GranuCult® prime Columbia Agar (base) acc. ISO 10272 and EP/USP/JP	1.00214.0500	500 g
GranuCult® prime Iron Sulfite Agar acc. ISO 15213-1	1.10864.0500	500 g
GranuCult® prime Tryptic Soy agar (TSA) acc. EP, USP, JP, ISO and FDA-BAM	1.05458.0500	500 g
GranuCult® prime Brain Heart Infusion (BHI) agar acc. FDA-BAM	1.03870.0500	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

MERCK