# Technical Data Sheet

# GranuCult® prime
# Iron sulfite Agar (ISA) acc. ISO 15213-1

## Ordering number: 1.10864.0500

For the isolation, presumptive identification and enumeration of sulfite-reducing *Clostridium* spp., including *C. perfringens*, from food, animal feed, environmental samples and samples from the primary production stage.
Iron sulfite Agar (ISA) acc. ISO 15213-1 is also known as Sulfite iron agar (SIA) and Iron sulphite agar.
This culture medium complies with the specifications given by EN ISO 15213-1:2023.

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 contains peptone (for example, enzymatic digest of casein) providing carbon, nitrogen, vitamins and amino acids. Sodium disulfite (sodium metabisulfite) is reduced to sulfide by the enzyme sulfite reductase, produced by clostridia. The sulfide will then precipitate as a black deposit in the presence of iron(III) ammonium citrate. Agar is the solidifying agent.

Iron sulfite Agar (ISA) acc. ISO 15213-1 has the same composition as Tryptose sulfite cycloserine agar (base) but without the cycloserine and contains a reduced concentration of sulfite. It has been demonstrated by MOSSEL et al. that the reduction of sulfite concentration to 0.05% improves the detection of sulfite-sensitive strains of *Clostridium* spp., especially *C. sporogenes*.

On Iron sulfite Agar (ISA) acc. ISO 15213-1, typical colonies of presumptive sulfite-reducing *Clostridium* spp. (SRC) appear black or grey to yellow-brown stained. As Iron sulfite Agar (ISA) acc. EN ISO 15213-1 is not selective for *Clostridium* spp., typical colonies need to be confirmed by anaerobic and, in parallel, no aerobic growth on Columbia blood agar or another nutrient-rich medium (e.g. Tryptone soya agar or Brain heart infusion agar).

## Typical Composition

| Specified by EN ISO 15213-1:2023 | | GranuCult® prime Iron sulfite Agar acc. ISO 15213-1 | |
|---|---|---|---|
| Peptone* | 15 g/l | Peptone* | 15 g/l |
| Enzymatic digest of soya | 5 g/l | Enzymatic digest of soya | 5 g/l |
| Yeast Extract | 5 g/l | Yeast Extract | 5 g/l |
| Sodium disulfite (sodium metabisulfite), anhydrous | 0.5 g/l | Sodium disulfite (sodium metabisulfite), anhydrous | 0.5 g/l |
| Iron(III) ammonium citrate** | 1.0 g/l | Iron(III) ammonium citrate** | 1.0 g/l |
| Agar | 9.0-18 g/l*** | Agar-Agar**** | 12 g/l |
| Water | 1000 ml/l | Water | n/a |
| pH at 25 °C | 7.6 ± 0.2 | pH at 25 °C | 7.6 ± 0.2 |

* For example, enzymatic digest of casein.
** This reagent should contain at least 150 g/kg of iron.
*** Depending on the gel strength of the agar.
**** Agar-Agar is equivalent to other different terms of agar.

## Preparation

Dissolve 38.5 g in 1 liter of purified water. Heat in boiling water and agitate frequently until completely dissolved. Autoclave (15 minutes at 121 °C). Cool the medium, mix well and use it according to the intended application.

If the medium is to be used immediately for poured plate technique, cool it to 44 °C to 47 °C in a water bath before use. Use the molten medium as soon as possible, it should not be retained for more than 4 h, as specified by EN ISO 11133.

The dehydrated medium is a granulate with beige color.

The prepared medium is clear to slightly turbid and yellowish to yellowish-brown. Due to the composition, precipitate may be visible in the prepared culture medium after sterilisation. This has no effect on the performance of the culture medium.

The pH value at 25 °C is in the range of 7.6 ± 0.2.

## Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Following the procedure given by EN ISO 15213-1, use the pour plate technique with the medium cooled to 44 °C to 47 °C, with 12 ml to 15 ml for Petri dishes with a diameter of 90 mm or 45 ml to 50 ml for Petri dishes with a diameter of 140 mm. Allow to solidify by leaving the Petri dishes standing on a cool horizontal surface.

After complete solidification, pour about 5 ml of the medium for 90 mm Petri dishes or 10 ml for 140 mm Petri dishes as overlay, to prevent the development of spreading colonies on the surface of the medium. Allow to solidify as specified above.

Incubate the inverted plates at (37 ± 1) °C in an anaerobic atmosphere for (48 ± 2) h.

Examine the plates for presumptive sulfite-reducing *Clostridium* spp.: Typical colonies, which show black or grey to yellow-brown staining on or in the medium, are counted.

NOTE: Longer incubation can result in excess blackening of the plates.

Upon removal of the plates from the anaerobic atmosphere, plates shall be counted within 30 min as the colour of the colonies can rapidly fade and disappear upon exposure to oxygen. If anaerobic jars are used, the plates should be checked jar by jar or in small portions if the incubation was performed in an anaerobic incubator.

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 count typical colonies.

For confirmation, follow the procedure given by EN ISO 15213-1 by streaking on two non-selective agar plates, e.g. Columbia blood agar or another nutrient-rich medium (e.g. Tryptone soya agar or Brain heart infusion agar). Several isolates can be streaked onto identified sectors of each of the two non-selective agar plates. Streaks should obtain well-isolated colonies.

From each pair of plates, one is incubated in an aerobic atmosphere and the other in an anaerobic atmosphere at (37 ± 1) °C for (20 ± 2) h.

Typical colonies are confirmed as follows:

- If growth from one typical colony occurs on the anaerobically incubated (blood) agar plate and not on the aerobically incubated Columbia blood agar plate, the colony belongs to the genus *Clostridia*. This colony and other colonies with the same morphology on Iron Sulfite Agar acc. ISO 15213-1 are counted as sulfite-reducing *Clostridium* spp.

- If growth occurs from one typical colony on both the anaerobically and aerobically incubated blood agar plates, the colony does not belong to the genus *Clostridia*. Therefore, this colony and other colonies with the same morphology on Iron Sulfite Agar acc. ISO 15213-1 cannot be counted as sulfite-reducing *Clostridium* spp.





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## 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 15213-1:2023, self-prepared medium can be stored in closed containers or tubes at (5 ± 3) °C for up to four weeks in the dark. Prior to use, the stored medium is melted completely and cooled down to 44 °C to 47 °C before use.

## Microbiological Performance

The performance test is in accordance with the current versions of EN ISO 11133 and EN ISO 15213-1.

**Test method: Performance testing of solid culture media - Quantitative and qualitative method (poured plate)**

| Function | Control strains | Incubation | Reference medium | Method of control | Expected results | Specified by |
|---|---|---|---|---|---|---|
| Productivity | *Clostridium perfringens* ATCC® 13124™ [WDCM 00007] | (48 ± 2) h/ (37 ± 1) °C anaerobic atmosphere | Iron sulfite Agar (ISA), batch already validated | Quantitative, pour plate technique | Recovery ≥ 70 %<br><br>black colonies | EN ISO 15213-1:2023 |
| | *Clostridium perfringens* ATCC® 12916™ [WDCM 00080] | | | | | |
| | *Clostridium perfringens* ATCC® 10543™ [WDCM 00174] | | | | | |
| Specificity | *Escherichia coli* ATCC® 8739™ [WDCM 00012] | (48 ± 2) h/ (37 ± 1) °C anaerobic atmosphere | – | Qualitative, pour plate technique | No limit<br><br>no blackening of colonies | |
| | *Escherichia coli* ATCC® 25922™ [WDCM 00013] | | | | | |

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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*Clostridium perfringens* WDCM 00174
on GranuCult® prime Iron sulfite Agar (ISA) acc. ISO 15213-1

## Literature

EN ISO International Standardisation Organisation. Microbiology of the food chain - Horizontal method for the detection and enumeration of *Clostridium* spp. - Part 1: Enumeration of sulfite-reducing *Clostridium* spp. by colony-count technique. EN ISO 15213-1:2023.

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.

Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. (2012): Iron sulfite agar. In: Handbook of Culture Media for Food and Water Microbiology, pp. 784-786. Royal Society of Chemistry, Cambridge, UK.

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.

Mossel, D.A.A., v. Golstein Brouwers, G.W.M. and de Bruin, A.S. (1959): A simplified method for the isolation and study of obligate anaerobes. J. Path. Bact. **78**, 290-291.

## Ordering Information

| Product | Cat. No. | Pack size |
|---|---|---|
| GranuCult® prime<br>Iron Sulfite Agar acc. ISO 15213-1 | 1.10864.0500 | 500 g |
| GranuCult® prime<br>TSC (Tryptose Sulfite Cycloserine) Agar (base)<br>acc. ISO 15213, ISO 14189 and FDA-BAM | 1.11972.0500 | 500 g |
| GranuCult® prime<br>Columbia Agar (base) acc. ISO 10272 and EP/USP/JP | 1.00214.0500 | 500 g |
| GranuCult® prime<br>Tryptic Soy agar (TSA) acc. EP, USP, JP, ISO and FDA-BAM | 1.05458.0500 | 500 g |
| GranuCult® prime<br>Brain Heart Infusion (BHI) agar acc. FDA-BAM | 1.03870.0500 | 500 g |
| GranuCult® prime<br>SIM (Sulfite Indole Motility) Agar acc. ISO 15213 | 1.05470.0500 | 500 g |
| Bactident® Indole (KOVÁCS Indole reagent)<br>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 |
| Anaerocult® P<br>Reagent for the generation of an anaerobic atmosphere<br>for one Petri dish | 1.32382.0001 | 25 x 1 set |
| Anaerocult® A mini<br>Gas generator system for the incubation<br>of one to four petri dishes in an anaerobic atmosphere | 1.32369.0001 | 25 x 1 set |
| Anaerocult® A<br>Reagent for the generation of an anaerobic atmosphere<br>in an anaerobic jar | 1.32381.0001 | 10 x 1 piece |
| Anaerotest®<br>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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