Technical Data Sheet

GranuCult® prime
SS (Salmonella Shigella) agar ref. to ISO 6579

Ordering number: 1.03853.0500

For the isolation and differentiation of *Salmonella* from food and animal feed, water and other materials.

This culture medium complies with the specifications given by APHA and reference to EN ISO 6579-1.

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 brilliant green, ox bile and high concentrations of thiosulfate and citrate which largely inhibit the accompanying microbial flora and swarming of *Proteus*, while allowing the growth of *Salmonella*. The combination of thiosulfate as a reactive compound with an iron salt as an indicator causes H_2S-positive colonies to become black in color. Lactose is incorporated for the differentiation between lactose-fermenting and non-fermenting bacteria. Lactose non-fermenting bacteria form colourless colonies. The presence of lactose-positive bacteria is established by detecting degradation of lactose to acid with the pH indicator neutral red. This results in the formation of red colonies by the lactose-fermenting bacteria. Peptones provide nitrogen, vitamins, minerals and amino acids essential for growth and agar acts as solidifying agent.

This culture medium is not recommended to be used for the primary cultivation of *Shigella*.

Typical Composition

EN ISO 6579-1 and APHA specify no composition for SS (Salmonella Shigella) agar.

GranuCult® prime SS (Salmonella Shigella) agar ref. to ISO 6579	
Peptones	10.0 g/l
Lactose	10.0 g/l
Ox bile, dried	8.5 g/l
Sodium citrate	8.5 g/l
Sodium thiosulfate	8.5 g/l
Ammonium iron(III) citrate	1.0 g/l
Brilliant green	0.00033 g/l
Neutral red	0.025 g/l
Agar-agar*	13.5 g/l
Water	n/a
pH at 25 °C	7.0 ± 0.2

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

Preparation

Dissolve 60.0 g in 1 liter of purified water. Heat in boiling water and agitate frequently until completely dissolved. Do not autoclave! Cool rapidly and pour plates to give a thick layer of medium.

The dehydrated medium is a granulate with re brown color.

The prepared medium is clear to slightly opalescent and reddish-brown. The pH value at 25 °C is in the range of 6.8 - 7.2.

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

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.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.
Following the procedure given by EN ISO 6579-1, and APHA, inoculate the surface of the medium from the selective enriched cultures so that well-isolated colonies will be obtained.

Incubate the inoculated plates inverted under aerobic conditions, e.g.

- acc. to EN ISO 6579-1 at (37 ± 1 °C) for (22 ± 2 h);
- acc. to APHA Chapter No. 36 at (35 ± 2 °C) for 16 h to 24 h.

On SS (Salmonella Shigella) agar ref. to ISO 6579, *Salmonella* spp. produce translucent colonies with or without black center and yellowish-brown coloured medium, or may appear as almost completely black colonies due to H_2S production.
Shigella spp. produce translucent colonies without black center and yellowish-brown coloured medium.
Lactose-fermenting Gram-negative bacteria may be inhibited or may produce red to pink colored colonies with pink-red coloured medium with or without precipitation zone.

Colonies of the most important bacteria usually have the appearance described below:

Appearance of colonies	**Microorganisms**
Colourless, translucent	*Shigella* and some *Salmonella* species
Translucent with a black center centre	*Proteus* and most *Salmonella* species
Pink to red	*Escherichia coli*
Colonies are larger than those of *Escherichia coli*, pink to whitish or cream-colored, opaque, mucoid	*Enterobacter* and *Klebsiella* species

This presumptive evidence must be confirmed by carrying out the usual tests.

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.

Self-prepared plates can be stored in the dark and protected against evaporation acc. to MacFaddin (1985) at (5 ± 3 °C) for up to 6-8 weeks.

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 testing

Test strain	Specification	
	Growth	Typical reaction
Shigella flexneri ATCC® 29903 [WDCM 00125]	good	translucent colonies without black center and yellowish-brown coloured medium
Salmonella Typhimurium ATCC® 14028 [WDCM 00031]	good	translucent colonies with black center and yellowish-brown coloured medium
Salmonella Enteritidis ATCC® 13076 [WDCM 00030]	good	translucent colonies with black center and yellowish-brown coloured medium
Proteus mirabilis ATCC® 29906 [WDCM 00023]	good	translucent colonies with black center and yellowish-brown coloured medium
Escherichia coli ATCC® 25922 [WDCM 00013]	growth or partial inhibition	if growth: pink colonies without black center and pink-red coloured medium
Staphylococcus aureus ATCC® 25923 [WDCM 00034]	none	-

Incubation: 22 ± 2 h at 37 ± 1 °C, aerobic.

Please refer to the actual batch related Certificate of Analysis.

Literature

APHA (2015) Chapter No. 36: *Salmonella*. and Chapter No. 67: Microbiological media, reagents and stains. Compendium of Methods for the Microbiological Examination of Foods. 5th ed. American Public Health Association, Washington, D.C.

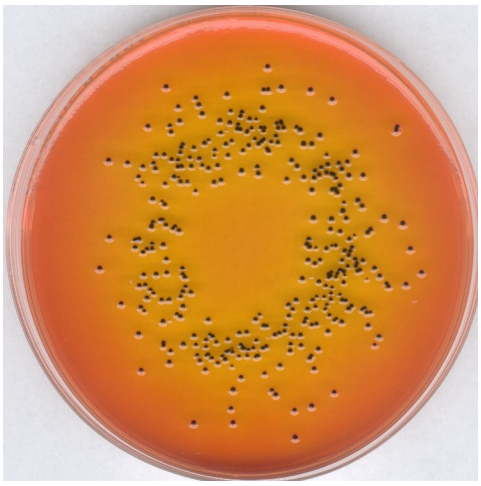
EN ISO International Standardisation Organisation. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* -
Part 1: Horizontal method for the detection of *Salmonella* spp. + Amendment 1. EN ISO 6579-1:2017/Amd1:2020.

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.

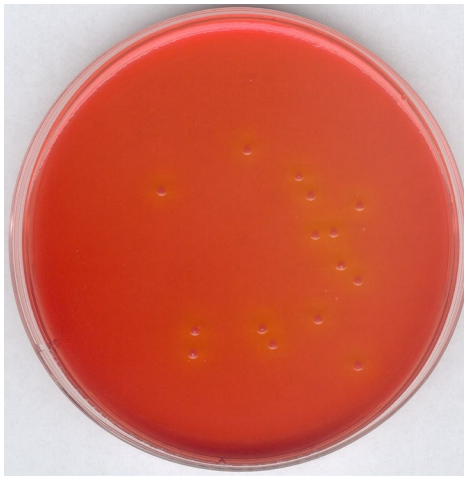
Leifson, E. (1935): New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Path. Bact. **40**:581-599.

MacFaddin, J.F. (1985): Media for isolation – cultivation – identification – maintenance of medical bacteria. Vol 1. 692 - 695. Williams & Wilkins, Baltimore, MD, USA.

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.



Salmonella Enteritidis
ATCC® 14028
[WDCM 00031]



Shigella flexneri
ATCC® 29903
[WDCM 00125]

Ordering Information

Product	Cat. No.	Pack size
GranuCult® prime SS (Salmonella Shigella) agar ref. to ISO 6579	1.03853.0500	500 g