

Technical Data Sheet

GranuCult® prime

SIMMONS citrate agar acc. ISO 10273 and FDA-BAM

Ordering number: 1.03855.0500

For the differentiation of Gram-negative bacteria, primary among the *Enterobacteriaceae*, from food and animal feed, water and other materials, based on citrate utilization.

This culture medium complies with the specifications given by EN ISO 10273, FDA-BAM Medium M138, AOAC Official Method 967.25, GB 4789.5, GB 4789.38, GB 4789.40, and APHA.

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 is used for the ability of an organism to utilize citrate as the sole source of carbon and inorganic ammonium salt as sole source of nitrogen for metabolism with resultant alkalinity. The alkaline reaction is evidenced by a change in the colour of the pH indicator bromothymol blue from green (neutral pH) to blue (alkaline pH).

Simmons citrate agar is a synthetic modification of Koser's medium with the addition of 1.5% agar and the pH indicator bromothymol blue.

Sodium citrate is the sole source of carbon and ammonium dihydrogen phosphate is the sole source of nitrogen. Magnesium sulfate is a cofactor for various metabolic reactions and sodium chloride maintains the osmotic balance. Bromothymol blue is the pH indicator and agar the solidifying agent.

Typical Composition

APHA specifies no composition for Simmons citrate agar.

Specified by EN ISO 10273		Specified by FDA-BAM Medium M138, AOAC 967.25		Specified by GB 4789.5, GB 4789.38, GB 4789.40		GranuCult® prime SIMMONS citrate agar acc. ISO 10273 and FDA-BAM	
Sodium citrate	2.0 g/l	Sodium citrate	2.0 g/l	Sodium citrate	2.0 g/l	Sodium citrate	2.0 g/l
NaCl	5.0 g/l	NaCl	5.0 g/l	NaCl	5.0 g/l	NaCl	5.0 g/l
K ₂ HPO ₄	1.0 g/l	K ₂ HPO ₄	1.0 g/l	K ₂ HPO ₄	1.0 g/l	K ₂ HPO ₄	1.0 g/l
Bromo-thymol blue	0.08 g/l	Bromo-thymol blue	0.08 g/l	Bromo-thymol blue	0.08 g/l	Bromo-thymol blue	0.08 g/l
Ammonium dihydrogen phosphate	1.0 g/l	Ammonium dihydrogen phosphate	1.0 g/l	Ammonium dihydrogen phosphate	1.0 g/l	Ammonium dihydrogen phosphate	1.0 g/l
Magnesium sulfate	0.2 g/l	Magnesium sulfate	0.2 g/l	Magnesium sulfate	0.2 g/l	Magnesium sulfate	0.2 g/l
Agar	12.0 g/l	Agar	15.0 g/l	Agar	8.0 g to 18.0 g/l*	Agar-agar**	13.0 g/l
Water	1000 ml	Water	1 liter	Water	1000 ml	Water	n/a
pH at 25 °C	6.8 ± 0.2	pH at 25 °C	6.8 ± 0.2	pH at 25 °C	6.8 ± 0.2	pH at 25 °C	6.8 ± 0.2

*Depending on the gel strength of the agar; GB 4789.5 specifies "Agar 20 g/l".

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

Preparation

Dissolve 22.5 g in 1 liter of purified water. Heat in boiling water and agitate frequently until completely dissolved. Autoclave (15 minutes at 121 °C). Pour plates or prepare slant agar tubes.

For the preparation of slant agar tubes, place tubes in slanted position before medium solidifies, so that long slants (approx. 4-5 cm, respectively) and short butts (approx. 2-3 cm, respectively) are formed on solidification.

The dehydrated medium is a granulate with yellow-greenish color.

The prepared medium is clear to slightly opalescent and green. The pH value at 25 °C is in the range of 6.6 - 7.0.

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

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Take a well isolated colony of a pure culture of the microorganisms to be tested and streak it on the surface of Simmons citrate agar plates.

Alternatively when using tubes, inoculate only the long slant (so called "fishtail slant") using a light inoculum from a pure culture in serpentine manner.

According to EN ISO 10273, take a well isolated colony of a strain to be tested and mix it well with a drop of saline solution. Streak the surface of Simmons citrate agar plates with the bacterial suspension. This "washing step" with saline removes excessive nutrients from the inoculum and thus helps to avoid false-positive results and interpretation problems.

Incubate the inoculated plates inverted and the tubes with loosen caps under aerobic conditions, e. g.

- acc. to EN ISO 10273 at (30 ± 1 °C) for (24 ± 2 h);
- acc. to FDA-BAM Chapter No. 5 and APHA Chapter No. 6 at (35 ± 2 °C) for (96 ± 2 h);
- acc. to GB 4789.5 at (36 ± 1 °C) for 4 days;
- acc. to GB 4789.38, GB 4789.40 at (36 ± 1 °C) for (24 ± 2 h).

The reaction is positive if there is visible growth on the medium and it turns from green to blue.

A light colour change to faint blue is also considered positive.

A negative reaction is evidenced by no growth (or very little trace growth) and no change in colour (green).

Usual growth and reaction of the most important bacteria as described below:

Growth and colour reaction	Microorganisms
Visible growth, medium turns from green to mostly deep blue, sometimes to faint blue	Citrate-positive: <i>Citrobacter</i> , <i>Enterobacter</i> , most <i>Salmonella</i> , <i>Klebsiella</i> , <i>Serratia</i> , <i>Yersinia enterocolitica</i> and others
No or mostly inhibited growth, medium remains green	Citrate-negative: <i>Escherichia</i> , <i>Shigella</i> , and others

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.

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 method for confirmation media and reagents.

Test strain	Specification (slant agar tube)
<i>Enterobacter cloacae</i> ATCC® 13047 [WDCM 00083]	growth colour change to blue
<i>Salmonella</i> Typhimurium ATCC® 14028 [WDCM 00031]	growth colour change to blue
<i>Klebsiella pneumoniae</i> ATCC® 13883 [WDCM 00097]	growth colour change to blue
<i>Yersinia intermedia</i> ATCC® 29909 [WDCM 00217]	growth light colour change to blue
<i>Escherichia coli</i> ATCC® 25922 [WDCM 00013]	none to weak growth no colour change to blue
<i>Shigella flexneri</i> ATCC® 12022 [WDCM 00126]	none to weak growth no colour change to blue
<i>Yersinia enterocolitica</i> DSM 13030 [WDCM 00216]	none to weak growth no colour change to blue
<i>Yersinia enterocolitica</i> ATCC® 23715 [WDCM 00160]	none to weak growth no colour change to blue

Incubation: 24 ± 2 h at 37 ± 1 °C, aerobic.

Yersinia spp. 24 ± 2 h at 30 ± 1 °C, aerobic.

Please refer to the actual batch related Certificate of Analysis.

Literature

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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.

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Ordering Information

Product	Cat. No.	Pack size
GranuCult® prime SIMMONS citrate agar acc. ISO 10273 and FDA-BAM	1.03855.0500	500 g

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