Technical Data Sheet

GranuCult® prime

KLIGLER agar acc. FDA-BAM

Ordering number: 1.03859.0500

For the confirmation of Gram-negative bacteria.

KLIGLER agar acc. FDA-BAM is also known as Kligler iron agar (KIA) and as Double sugar iron agar (Kligler).

This culture medium complies with the specifications given by FDA-BAM Medium M71.

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 differentiation of Gram-negative bacteria, mainly *Enterobacteriaceae*, on the base of their ability to ferment glucose and lactose (carbohydrate fermentation) and to release sulfides (H_2S production).

KLIGLER agar acc. FDA-BAM contains two carbohydrates: 1.0% lactose and 0.1% glucose (dextrose). In this medium some organisms can ferment both carbohydrates, others ferment only glucose; still others can ferment neither lactose nor glucose. Carbohydrate fermentation may occur with or without gas production. Fermentation occurs both aerobically (on the slant) and anaerobically (in the butt). The colour changes of the pH indicator phenol red is in response to the acid produced during this fermentation of the sugars.

Another system for the differentiation is provided by the H_2S indicators in the medium: ferric ammonium citrate and sodium thiosulfate. A black precipitate of ferrous sulfide indicates H_2S production – it may mask the acid condition produced in the butt of the medium, Therefore, if H_2S is produced, an acid condition exists even if not observable.

Gas production (aerogenic reaction) is detected as individual bubbles or by splitting or displacement of the agar.

Nitrogen, carbon, and vitamins required for organism growth are provided by peptones, sodium chloride maintains the osmotic balance of the medium and agar is the solidifying agent.

Typical Composition

Specified by FDA-BAM Medium M71		GranuCult® prime KLIGLER agar acc. FDA-BAM	
Polypeptone peptone	20.0 g/l	Peptones	20.0 g/l
NaCl	5.0 g/l	NaCl	5.0 g/l
Lactose	10.0 g/l	Lactose	10.0 g/l
Dextrose	1.0 g/l	D(+)Glucose*	1.0 g/l
Ferric ammonium citrate	0.5 g/l	Ammonium iron(III) citrate**	0.5 g/l
Sodium thiosulfate	0.5 g/l	Sodium thiosulfate	0.5 g/l
Phenol red	0.024 g/l	Phenol red	0.024 g/l
Agar	15.0 g/l	Agar-agar***	18.0 g/l
Water	1 liter	Water	n/a
pH at 25 °C	7.4 ± 0.2	pH at 25 °C	7.4 ± 0.2

*D(+)-Glucose is equivalent to the term Dextrose.
** Ammonium iron(III) citrate is equivalent to the term Ferric ammonium citrate.
***Agar-Agar is equivalent to other different terms of agar.

Preparation

Dissolve 55.0 g in 1 liter of purified water. Heat in boiling water and agitate frequently until completely dissolved. Dispense into tubes and autoclave (15 minutes at 121 °C). Allow to solidify to give agar slants with deep butts.

The dehydrated medium is a granulate with reddish-brown color.

The prepared medium is clear to slightly opalescent and red. The pH value at 25 °C is in the range of 7.2 - 7.6.

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 material from a well isolated colony of a pure culture of the microorganisms to be tested and inoculate the long slant (so called "fishtail slant") in serpentine manner and stab the butt. Inoculation order may be reversed if preferred.

Incubate the tubes 18 to 24 h at 35 ± 1 °C, aerobic with loosen caps.

An alkaline slant-acid butt (red/yellow) indicates fermentation of glucose (dextrose) only.

An acid slant-acid butt (yellow/yellow) indicates fermentation of glucose (dextrose) and lactose.

An alkaline slant-alkaline butt (red/red) indicates dextrose and lactose did not ferment (non-fermenter).

Cracks, splits, or bubbles in the medium indicate gas production.

Blackening in the butt (and may be as well in the slant) indicates hydrogen sulfide production.

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		
	Growth	Typical reaction	
		Butt	Slant
Escherichia coli ATCC® 25922 [WDCM 00013]	good	yellow	yellow
Citrobacter freundii ATCC® 8090	good	yellow and black	yellow
Shigella flexneri ATCC® 12022 [WDCM 00126]	good	yellow	red
Salmonella Typhimurium ATCC® 14028 [WDCM 00031]	good	yellow and black	red
Salmonella Enteritidis ATCC® 13076 [WDCM 00030]	good	yellow and black	red
Proteus mirabilis ATCC® 14153	good	yellow	red
Proteus hauseri ATCC® 13315	good	yellow and black	red

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

Please refer to the actual batch related Certificate of Analysis.

Literature

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.

FDA-BAM (2018): Media Index for BAM - BAM Media M71: Kligler Iron Agar. Food and Drug Administration - Bacteriological Analytical Manual.

Kligler, I.J. (1917): A simple medium for the differentiation of embers of the typhoid-paratyphoid group. Am. J. Public Health, **7**: 1042-1044.

MacFaddin, J.F. (1985): Media for isolation – cultivation – identification – maintenance of medical bacteria. Vol 1. 398-405. Wiliams & Wilkins, Baltimore, MD, USA.

MacFaddin, J.F. (2000): Biochemical test for identification of medical bacteria. pp. 239-253. 3rd ed. Lippincott, Wiliams & Wilkins, Baltimore, MD, USA.

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1 *Escherichia coli* ATCC® 25922 [WDCM 00013]

2 *Citrobacter freundii* ATCC® 8090

3 *Shigella flexneri* ATCC® 12022 [WDCM 00126]

4 *Salmonella* Typhimurium ATCC® 14028 [WDCM 00031]

5 *Proteus mirabilis* ATCC® 14153

on KLIGLER agar acc. FDA-BAM (from left to right)

Ordering Information

Product	Cat. No.	Pack size
GranuCult® prime KLIGLER agar acc. FDA-BAM	1.03859.0500	500 g