

Technical Data Sheet

GranuCult® plus DHL (Deoxycholate Hydrogen sulfide Lactose) agar acc. to SAKAZAKI

Ordering number: 1.03867.0500

For the isolation and differentiation of Gram-negative bacteria from different materials.

DHL (Deoxycholate Hydrogen sulfide Lactose) agar acc. to SAKAZAKI is also known as Sakazaki DHL agar.

Mode of Action

This culture medium is a modification of Deoxycholate Agar and was proposed by Sakazaki et al. (1960, 1971).

H₂S production is indicated by a blackening of the colonies due to formation of iron sulfide. Although *Proteus* is H₂S-positive, its colonies are not black.

Colonies of *Proteus*, *Morganella*, *Rettingerella* and *Providencia* are, however, surrounded by dark brown zones, which occur, because these species act on the phenylalanine of the peptone to produce phenylpyruvate, which forms an iron complex with iron(III) ions.

The sucrose content of the medium permits differentiation of weakly lactose-positive or lactose-negative, sucrose-positive species from sucrose- and lactose-negative Enterobacteriaceae.

The deoxycholate largely suppresses the growth of Gram-positive bacteria and prevents the swarming of *Proteus* species. This medium provides a rich nutrient base and contains a relatively low concentration of the inhibitor deoxycholate. These properties permit growth of even fastidious strains of *Salmonella* and *Shigella*. The colonies formed are considerably larger than those found on other selective culture media. *Proteus*, *Morganella*, *Rettingerella* and *Providencia* can be differentiated from *Salmonella*.

Neutral Red is a pH indicator and agar is the solidifying agent.

Typical Composition

GranuCult® plus DHL (Deoxycholate Hydrogen sulfide Lactose) agar acc. to SAKAZAKI	
Enzymatic digest of casein	10.0 g/l
Enzymatic digest of animal tissue	10.0 g/l
Meat extract	3.0 g/
Lactose	10.0 g/
Sucrose	10.0 g/l
L-Cystein hydrochloride	0.2 g/l
Sodium citrate	1.0 g/l
Sodium desoxycholate	1.5 g/l
Sodium thiosulfate	2.0 g/l
Ammonium iron(III) citrate	1.0 g/l
Neutral red	0.03 g/l
Agar-agar*	15.0 g/l
Water	n/a
pH at 25 °C	7.2 ± 0.2

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

Preparation

Dissolve 64.0 g in 1 liter of purified water. Heat in boiling water and agitate frequently until completely dissolved. Pour to plates to give thick layers (about 20 ml per Petri dish). Do not autoclave!

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

The prepared medium is clear to slightly opalescent and red. The pH value at 25 °C is in the range of 7.0 - 7.4.

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.

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Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Spread the sample or material preferable from an enrichment culture thinly on the surface of the plates.

Incubate at (35 ± 1 °C) or at (37 ± 1 °C) for 24-48 hours at 35 °C under aerobic conditions.

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

Test method: Performance testing of solid culture media - Qualitative testing

Test strain	Specification	
	Growth	Typical reaction
<i>Escherichia coli</i> ATCC® 25922 [WDCM 00013]	good	red colonies with precipitation halo
<i>Salmonella</i> Enteritidis ATCC® 13076 [WDCM 00030]	good	colourless colonies with black centre
<i>Salmonella</i> Typhimurium ATCC® 14028 [WDCM 00031]	good	colourless colonies with black centre
<i>Shigella flexneri</i> ATCC® 12022 [WDCM 00126]	weak to good	colourless colonies without black centre
<i>Proteus mirabilis</i> ATCC® 14153	good	colourless colonies without black centre, surrounded by a brownish zone
<i>Klebsiella pneumoniae</i> ATCC® 10031	good	pink colonies
<i>Bacillus cereus</i> ATCC® 11778 [WDCM 00001]	no growth	not applicable

Incubation: 22 ± 2 h at 35 ± 1 °C, aerobic.

Please refer to the actual batch related Certificate of Analysis.



Proteus mirabilis
ATCC® 14153
on DHL agar acc. to SAKAZAKI



Salmonella Enteritidis
ATCC® 13076 [WDCM 00030]
on DHL agar acc. to SAKAZAKI



Shigella flexneri
ATCC® 12022 [WDCM 00126]
on DHL agar acc. to SAKAZAKI

Ordering Information

Product	Cat. No.	Pack size
GranuCult® plus DHL (Deoxycholate Hydrogen sulfide Lactose) agar acc. to SAKAZAKI	1.03867.0500	500 g