

Kligler-Hajna/Agar (Lactose-Glucose-H₂S)

355-5378
356-4844

DEFINITION

Medium used for the identification of *Enterobacteria* following their isolation in food products and water.

STANDARDS

FOOD MICROBIOLOGY

- **NF EN ISO 10273 (December 2003):** Microbiology - Horizontal method for the detection of presumptive pathogenic *Yersinia enterocolitica*

WATER

- **ISO 19250 (July 2010):** Water quality - Detection and enumeration of *Salmonella*
- **NF T90-461/A2 (May 2007):** Water quality - Microbiology - Quality control for culture media

PRINCIPLE

The principle of the medium relies on the ability or otherwise of *Enterobacteria* to ferment lactose (inclined surface turns yellow = lactose + colonies) and glucose (yellow sediment = glucose + colonies) with or without production of gas or hydrogen sulfide (blackening).

PRESENTATION

- **Ready to use**
10 ml x 25 inclined tubes **code 355-5378**
- **Dehydrated**
500 g **code 356-4844**

STORAGE

- Ready to use: +2-8°C.
- Dehydrated: +15-25°C, in carefully-sealed bottles in a cool, dry place
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

Peptone	20 g
Bovine meat extract	3 g
Yeast extract	3 g
Sodium chloride	5 g
Ferric sulfate	0.2 g
Sodium thiosulfate	0.3 g
Lactose	10 g
Glucose	1 g
Phenol red	25 mg
Agar	11 g
Distilled water	1,000 ml

Final pH (25°C) = 7.5 ± 0.2

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)

- Distilled water

EQUIPMENT REQUIRED (NOT SUPPLIED) (non-exhaustive)

- Hotplate
- Mixer-homogenizer
- Test tubes (16 x 160 mm) with autoclave-proof stoppers
- Sterile Pasteur pipettes (code 355-0751) or inoculating loop
- Water-bath at 100°C for melting the ready-to-use media
- Thermostatically-controlled incubator or incubating room, precise to ±1°C
- Autoclave
- All usual laboratory equipment

PREPARATION OF DEHYDRATED MEDIUM

Always shake before use.

Dissolve 53.5 g of powder in 1 liter of distilled water. Bring to boiling point until a homogenous suspension is obtained.

Mix well and dispense 10 ml per tube. Sterilize in autoclave at 121°C ± 1°C for 15 minutes.

Leave to cool in an inclined position so as to obtain a pellet about 3 cm deep and a slope.

Once the surface of the slope is dry, the medium is ready-to-use.

NB: If this medium is prepared more than 8 days before use, it should preferably be melted in a boiling water-bath, then solidified again in the correct position.

**Reconstitution ratio: 53.5 g/l
500 g of powder makes 9.3 liters of medium.**

PROTOCOL

Inoculation and incubation

Inoculate the pellet by injection and the inclined surface by close parallel streaks, so as to obtain a film culture.

Incubate at 37°C ± 1°C for 18-24 hours.

Do not tighten the caps.

READING AND INTERPRETATION

This medium gives 3 responses in a maximum of 24 hours:

- **Fermentation of lactose:** the inclined surface turns yellow. If this is not the case, the color remains unchanged.
- **Fermentation of glucose:** the pellet turns yellow. If this is not the case, its color remains unchanged. If gas is produced, it is possible to observe either just a few bubbles or a pocket of gas lifting the medium completely off the base of the tube.
- **Production of H₂S:** blackening of the medium in the zone connecting pellet to slope. With bacteria producing little H₂S (*S.typhi*), the blackening remains localized at the injection site.

From the results obtained and other complementary tests, identification can take place with the aid of the following table:

Enterobacteriaceae	Gas Glucose	Lactose	ONPG	H ₂ S	LDC
<i>Salmonella SE</i> in general	+	-	-	+	+
<i>S. typhi</i>	-	-	-	+ weak	+
<i>S. paratyphi A</i>	+	-	-	-	-
<i>S. arizona SE III</i>	+	-	+	+	+
<i>Citrobacter</i>	+	d	+	+	+
<i>Edwardsiella</i>	+	-	-	+	+
<i>Escherichia coli</i>	+	d	+	-	d
<i>Alkalescens</i>	-	-	d	-	d
<i>Sh. dysenteriae</i>	-	-	d	-	-
<i>Sh. boydii, flexneri</i>	-	-	-	-	-
<i>Sh. sonnei</i>	-	-	d	-	-
<i>Proteus vulgaris</i>	+	-	-	+	-
<i>Proteus mirabilis</i>	+	-	-	+	-
<i>Proteus rettgeri</i>	d	-	-	-	-

PERFORMANCES/QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains:

Enterobacteriaceae	Gas Glucose	Lactose	ONPG	H ₂ S	LDC
<i>Proteus morganii</i>	+	-	-	-	-
<i>Providencia</i>	d	-	-	-	-
<i>Levinea</i>	+	-	+	-	-
<i>Y. enterocolitica</i>	-	-	+	-	-
<i>Y. pseudotuberculosis</i>	-	-	d	-	-
<i>K. pneumoniae</i>	+	+	+	-	+
<i>K. oxytoca</i>	+	+	+	-	+
<i>E. aerogenes</i>	+	d	+	-	+
<i>K. ozonae</i>	d	d	+	-	d
<i>K. rhinoscleromatis</i>	-	-	-	-	-
<i>E. cloacae</i>	+	-	+	-	-
<i>E. agglomerans</i>	d	d	+	-	-
<i>Hafnia alvei</i>	+	-	+	-	+
<i>Serratia arcscens and liquefaciens</i>	d	-	+	-	+
<i>V. parahemolyticus</i>	-	-	-	-	+

STRAINS	Results of 24-48 hr culture at 37°C			
	Glu	Lac	Gas	H ₂ S
<i>Escherichia coli</i> RIVM WR1 / CIP106878	+	+	+	-
<i>Klebsiella pneumoniae</i> ATCC 13883	+	+	+	-
<i>Citrobacter freundii</i> ATCC 8090	+	+	+	+
<i>Salmonella Enteritidis</i> ATCC 13076	+	-	+	+
<i>Shigella sonnei</i> ATCC 25931	+	-	-	-
<i>Proteus vulgaris</i> ATCC 6380	+	-	±	+
<i>Pseudomonas aeruginosa</i> ATCC 27853	-	-	-	-

QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

Kligler-Hajna/Agar

(Lactose-Glucose-H₂S)

V5 - 05/08/11

KEY WORDS

Kligler-Hajna/*Enterobacteriaceae*/Food products/Water/Identification/Isolation/Lactose/Glucose/H₂S/Gas/Fermentation/Medium

BIBLIOGRAPHY

CHATELAIN et Coll. (1979): Ann. Inst. Pasteur
130 A : 449-454