

KLIGLER IRON AGAR

For the differentiation of Gram negative Enterobacteria

CAT N°: 21042

FORMULA IN g/l

Peptone Mixture	20.00	Ferric Citrate	Ammonium	0.50
Lactose	10.00	Sodium Thiosulfate		0.50
Sodium Chloride	5.00	Phenol Red		0.025
Dextrose	1.00	Bacteriological Agar		15.00

Final pH 7.4 ± 0.2 at 25°C

PREPARATION

Suspend 52 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes and sterilize in autoclave at 121°C for 15 minutes. Allow to cool in a slanted position in order to obtain butts of 1.5-2 cm depth. The prepared medium should be stored at 2-8°C. The color is pink-orange. For best results, Kligler Iron Agar should be used on the day of preparation or melted and solidified before use.

The dehydrated medium should be homogeneous, free-flowing and beige-pink in color. If there are any physical changes, discard the medium.



Uninoculated Tube



Salmonella enteritidis
ATCC 13076

USES

KLIGLER IRON AGAR may be used to differentiate Gram-negative Enterobacteria on the basis of carbohydrate fermentation and H₂S production.

The Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Dextrose and Lactose are the fermentable carbohydrates, producing acid indicated by the Phenol red indicator. The color changes that result are yellow for acid production and red for alkalization. Sodium thiosulfate is reduced to hydrogen sulfide, which reacts with the iron salt to give the black iron sulfide. Sodium sulfide and Ferric ammonium citrate are H₂S indicators. Bacteriological agar is the solidifying agent.

Inoculate the medium with the colony under study by stabbing the butt and streaking the surface of the tube. Incubate at 35 ± 2°C for 24 hours. Lactose non fermenters (e.g., *Salmonella* and *Shigella*) initially produce a yellow slant due to acid formation caused by the fermentation of dextrose. Once the dextrose supply runs out in the aerobic environment of the slant, the reaction reverts to alkaline (red slant) due to oxidation of the acids. The reversion does not occur in the anaerobic environment in the butt, which remains acid (yellow butt). Lactose fermenters produce yellow slants and butts due to the fact that sufficient acid is produced in the slant to maintain an acid pH under aerobic conditions. Organisms incapable of fermenting the carbohydrates produce red slants and butts.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 hours.

Microorganisms	Growth	Slant	Base	H ₂ S	G a s
<i>Escherichia coli</i> ATCC 25922	Good	Yellow	Yellow	-	+
<i>Proteus vulgaris</i> ATCC 6380	Good	Red	Yellow	+	-
<i>Salmonella enteritidis</i> ATCC 13076	Good	Red	Yellow	+	+
<i>Shigella flexneri</i> ATCC 12022	Good	Red	Yellow	-	-
<i>Citrobacter freundii</i> ATCC 8090	Good	Yellow	Yellow	+	+

BIBLIOGRAPHY

J. Bact. 13:1 83. 1927. J. Bact. Clin. Med. 25:649, 1940.



STORAGE

Once opened keep powdered medium closed to avoid hydration.

