# CM 22,740 - KLIGLER IRON AGAR SLANT

## INTENDED USE

For differentialidentification of Gram-negative enteric bacilli on the basis of the fermentation of dextrose, lactose and H2S Production.

## PRODUCTSUMMARY AND EXPLANATION

Kligler Iron Agar is a combination of theleadacetate medium described by Kligler and Russels Double Sugar Agar and is used as a differentiation medium for typhoid,dysentery and allied bacilli. Bailey and Lacey substituted phenol red for andrade indicator previously used as pH indicator. Kligler Iron Agar differentiates lactose fermenters from the non-fermenters. It differentiates *Salmonella typhi*from other Salmonellae and also *Salmonella paratyphi* A from *Salmonella scottmuelleri* and *Salmonella enteritidis*. Fermentation of dextrose results in production of acid, which turns the indicator from red to yellow. Since there is little sugari.e.dextrose, acid production is very limited and therefore a reoxidation of the indicator is produced on the surface ofthe medium, and the indicator remains red. However, when lactose is fermented, the large amount of acid produced,avoids reoxidation and therefore the entire medium turns yellow.

## COMPOSITION

| Ingredients | Gms / Ltr |
|---|---|
| **Agar** | 15.000 |
| **Peptone** | 15.000 |
| **Lactose** | 10.000 |
| **Proteose peptone** | 5.000 |
| **Sodium chloride** | 5.000 |
| **Beef extract** | 3.000 |
| **Yeast extract** | 3.000 |
| **Dextrose** | 1.000 |
| **Sodium thiosulphate** | 0.300 |
| **Ferrous sulphate** | 0.200 |
| **Phenol red** | 0.024 |

## PRINCIPLE

Kligler Iron Agar, inaddition topeptone, Beef extract and yeast extract, contains lactose and glucose (dextrose), which enables the differentiationof species of enteric bacilli. Phenol red is the pH indicator, which exhibits a colour change in response to acid produced during the fermentation of sugars. The combination of ferrous sulphate and sodium thiosulphate enablesthedetection of hydrogen sulfide production, which is evidenced by a black color either throughout the butt, or in a ringformation near the top of the butt. Lactose non-fermenters (e.g., *Salmonella* and *Shigella*) initially produce a yellow slantdueto acid produced by the fermentation of the small amount of glucose (dextrose). When glucose (dextrose) supply isexhausted in the aerobic environment of the slant, the reaction reverts to alkaline (red slant) due to oxidation of the acidsproduced. The reversion does not occur in the anaerobic environment of the butt, which therefore remains acidic (yellowbutt). Lactose fermenters produce yellow slants and butts because of lactose fermentation. The high amount of acidsthusproduced helps to maintain an acidic pH under aerobic conditions. Tubes showing original colour of the mediumindicates the fermentation of neither glucose (dextrose) nor lactose. Gas production (aerogenic reaction) is detectedasindividual bubbles or by splitting or displacement of the agar by the formation of cracks in the butt of the medium.

## INSTRUCTION FORUSE

Inoculatethebacterialculture with an inoculating needle by streaking the slants.

## QUALITY CONTROL SPECIFICATIONS

**Appearance** : Red coloured, clear to slightly opalescent gel forms in tubes as slants

**Quantity of Medium** : 8 ml of medium in glass tube.

**pH ( at 25°C)** : 7.4±0.2

## INTERPRETATION

Culturalcharacteristics observed after an incubation.

| Microorganism | ATCC | Growth | Gas | $H_2S$ | Slant | Butt | Incubation Temperature | Incubation Time |
|---|---|---|---|---|---|---|---|---|
| *Escherichia coli* | 25922 | Luxuriant | Positive reaction | Negative reaction, no blackening of medium | Acidic reaction, yellowing of the medium | Acidic reaction, yellowing of the medium | 35-37°C | 18 - 48 hours. |
| *Klebsiella aerogenes* | 13048 | Luxuriant | Positive reaction | Negative reaction, no blackening of medium | Acidic reaction, yellowing of the medium | Acidic reaction, yellowing of the medium | 35-37°C | 18 - 48 hours. |
| *Citrobacter freundii* | 8090 | Luxuriant | Positive reaction | Positive reaction, blackening of medium | Acidic reaction, yellowing of the medium | Acidic reaction, yellowing of the medium | 35-37°C | 18 - 48 hours. |
| *Proteus vulgaris* | 6380 | Luxuriant | Negative reaction | Positive reaction, blackening of medium | Alkaline reaction, red colour of the medium | Acidic reaction, yellowing of the medium | 35-37°C | 18 - 48 hours. |
| *Klebsiella pneumoniae* | 13883 | Luxuriant | Positive reaction | Negative reaction, no blackening of medium | Acidic reaction, yellowing of the medium | Acidic reaction, yellowing of the medium | 35-37°C | 18 - 48 hours. |
| *Salmonella paratyphi A* | 9150 | Luxuriant | Positive reaction | Negative reaction, no blackening of medium | Alkaline reaction, red colour of the medium | Acidic reaction, yellowing of the medium | 35-37°C | 18 - 48 hours. |
| *Salmonella typhi* | 6539 | Luxuriant | Negative reaction | Positive reaction, blackening of medium | Alkaline reaction, red colour of the medium | Acidic reaction, yellowing of the medium | 35-37°C | 18 - 48 hours. |
| *Salmonella enteritidis* | 13076 | Luxuriant | Positive reaction | Positive reaction, blackening of medium | Alkaline reaction, red colour of the medium | Acidic reaction, yellowing of the medium | 35-37°C | 18 - 48 hours. |
| *Shigella flexneri* | 12022 | Luxuriant | Negative reaction | Negative reaction, no blackening of medium | Alkaline reaction, red colour of the medium | Acidic reaction, yellowing of the medium | 35-37°C | 18 - 48 hours. |
| *Pseudomonas aeruginosa* | 27853 | Luxuriant | Negative reaction | Negative reaction, no | Alkaline reaction, red colour of the medium | Alkaline reaction, red colour of the medium | 35-37°C | 18 - 48 hours. |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | blackening of medium | | | | |
| *Yersinia enterocolitica* | 27729 | Luxuriant | Variable reaction | Negative reaction, no blackening of medium | Alkaline reaction, red colour of the medium | Acidic reaction, yellowing of the medium | 35-37°C | 18 - 48 hours. |
| *Enterobacter cloacae* | 13047 | Luxuriant | Positive reaction | Negative reaction, no blackening of medium | Acidic reaction, yellowing of the medium | Acidic reaction, yellowing of the medium | 35-37°C | 18 - 48 hours. |

**PACKAGING:**

Kitof10Ready-To-Use Slants containing 8 ml medium in each glass tube.

**STORAGE**

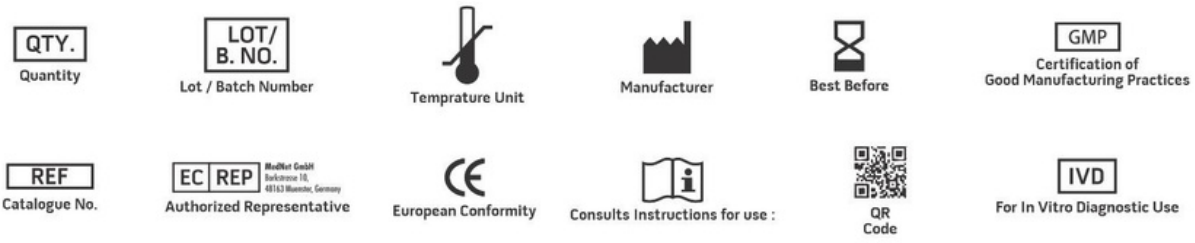
Onreceipt, store tubes in the dark at 2 – 8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation. **Product Deterioration:** Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

**DISPOSAL**

Usermustensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

**REFERENCES**

1. Bailey S. F. and Lacey G. R., 1927, J. Bacteriol., 13:183.
2. Ewing, 1986, Edwards and Ewings Identification of the Enterobacteriaceae, 4th Ed., Elsevier Science Publishing Co., Inc., N.Y.
3. Kligler I. J., 1917, Am. J. Publ. Health, 7:1041.
4. Russell F. F., 1911, J. Med. Res., 25:217.




**NOTE:** Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**