KLIGLER IRON AGAR (KIA)

Cat. no. L70	KIA Slant, 16x125mm Tube, 8ml Slant	20 or 100 tubes/box
Cat. no. R70	KIA Slant, 13x100mm Tube, 4.5ml Slant	20 or 100 tubes/box

INTENDED USE

Hardy Diagnostics Kligler Iron Agar (KIA) is recommended for use in differentiating certain members of the Enterobacteriaceae by demonstrating hydrogen sulfide production and the fermentation of dextrose and lactose.

SUMMARY

Kligler Iron Agar (KIA) combines features of Kligler's Lead Acetate medium and Russell's Double Sugar Agar. [8,9] Phenol red is added as the color indicator. The basal medium of KIA is composed of casein and meat peptones with the addition of lactose and dextrose. The production of acid by lactose and/or dextrose fermentation results in color changes of the phenol red pH indicator. Presence of the carbohydrates thus enables the differentiation of species of enteric bacilli.

Non-lactose fermenters initially produce a yellow slant and butt as a result of dextrose fermentation. The concentration of dextrose is only one percent and, therefore, is rapidly exhausted. Once the dextrose is depleted, the reaction reverts to alkaline (red slant) due to the oxidation of acids. Reversion does not occur in the butt of the medium where an acidic environment (yellow butt) is maintained. Lactose fermenting organisms produce yellow slants and butts. There is no reversion to red in the slant because enough acid is produced to maintain an acid pH under aerobic conditions. Non-fermenters produce red slants and butts. H_2S production results in a blackening of the medium, either throughout the butt or in a ring formation near the top of the butt. Gas production is demonstrated by the presence of bubbles or cracks in the medium.

FORMULA

Ingredients per liter of deionized water:*

Peptone	15.0gm
Lactose	10.0gm
Proteose Peptone	5.0gm
Sodium Chloride	5.0gm
Beef Extract	3.0gm
Yeast Extract	3.0gm
Dextrose	1.0gm

Sodium Thiosulfate	0.3gm
Ferrous Sulfate	0.2gm
Phenol Red	0.024gm
Agar	12.0gm

Final pH 7.4 +/- 0.2 at 25ºC.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-30ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration dating on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended quality control incubation times.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

Refer to the document SDS Search instructions on the Hardy Diagnostics' website for more information.

PROCEDURE

Specimen Collection: This product is not intended for primary isolation of patient specimens. It should be used only with cultures of isolated organism. This product is used in conjunction with other biochemical tests to identify cultures of isolated organism. Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation.

1. Allow KIA to warm to room temperature prior to inoculation.

2. Obtain a pure culture of the organism to be tested. Select well-isolated colonies.

3. With an inoculating needle, pick the cener of well-isolated colonies obtained from solid culture media.

4. Stab the center of the medium into the deep of the tube to within 3-5mm from the bottom.

5. Withdraw the inoculating needle and streak the surface of the slant.

6. Loosen closure on the tube before incubating.

7. Incubate aerobically at 35ºC. for 18-48 hours.

8. Read tubes for acid production of the slant/butt, gas, and hydrogen sulfide reactions.

INTERPRETATION OF RESULTS

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

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

An alkaline slant-alkaline butt (red/red) indicates that neither dextrose nor lactose was fermented (non-fermenter).

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

A black precipitate in the butt indicates hydrogen sulfide production.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.

It is important to stab the butt of the medium. Failure to stab the butt invalidates this test. The integrity of the agar must be maintained when stabbing. Caps must be loosened during this test or erroneous results will occur.

An organism that produces hydrogen sulfide may mask acid production in the butt of the medium. However, hydrogen sulfide production requires an acid environment, thus the butt portion should be considered acid if hydrogen sulfide is produced.

Certain species or strains may give delayed reactions or completely fail to ferment the carbohydrate in the stated manner. However, in most cases, if the organism fails to ferment dextrose within 48 hours and growth is definitely present, the organism is most likely not in the Enterobacteriaceae family.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results

		Time	Temperature	Atmosphere	
Salmonella enterica ATCC ® 14028	C	18-24hr	35°C	Aerobic	Growth; red slant, yellow butt, black butt, H_2S positive, gas positive
Escherichia coli ATCC ® 25922	C	18-24hr	35°C	Aerobic	Growth; yellow slant, yellow butt, H_2S negative, gas positive
Pseudomonas aeruginosa ATCC ® 27853	C	18-24hr	35°C	Aerobic	Growth; red slant, red butt, H_2S negative, gas negative

* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificates of analysis (CofA) available from Hardy Diagnostics Certificates of Analysis website. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see reference(s) for more specific information.

PHYSICAL APPEARANCE

Kligler Iron Agar should appear slightly opalescent, and orange-red in color.

Salmonella enterica (ATCC ® 14028) growing in Kligler Iron Agar (Cat. no. L70). Incubated aerobically for 24 hours at 35ºC.

Escherichia coli (ATCC ® 25922) growing in Kligler Iron Agar (Cat. no. L70). Incubated aerobically for 24 hours at 35ºC.

Pseudomonas aeruginosa (ATCC ® 27853) growing in Kligler Iron Agar (Cat. no. L70). Incubated aerobically for 24 hours at 35ºC.

Uninoculated tube of Kligler Iron Agar (Cat. no. L70).

REFERENCES

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

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