



# Instructions for Use

## CRITERION™ KLIGLER IRON AGAR (KIA)

Cat. no. C5910	CRITERION™ Kligler Iron Agar (KIA)	104gm
Cat. no. C5911	CRITERION™ Kligler Iron Agar (KIA)	500gm
Cat. no. C5912	CRITERION™ Kligler Iron Agar (KIA)	2kg
Cat. no. C5913	CRITERION™ Kligler Iron Agar (KIA)	10kg
Cat. no. C5914	CRITERION™ Kligler Iron Agar (KIA)	50kg

#### INTENDED USE

Hardy Diagnostics CRITERION™ 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.

This dehydrated culture medium is a raw material intended to be used in the making of prepared media products, which will require further processing, additional ingredients, or supplements.

## **SUMMARY**

CRITERION<sup>TM</sup> 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**

Gram weight per liter:	52.0gm/L
Pancreatic Digest of Casein	10.0gm
Peptic Digest of Animal Tissue	10.0gm

Lactose	10.0gm
Sodium Chloride	5.0gm
Dextrose	1.0gm
Sodium Thiosulfate	0.5gm
Ferric Ammonium Citrate	0.5gm
Phenol Red	25.0mg
Agar	15.0gm

Final pH 7.4 +/- 0.2 at 25°C.

## STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-30°C. Dehydrated culture medium is very hygroscopic. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. The dehydrated culture media should be discarded if it is not free-flowing or if the color has changed from its original pinkish-beige.

Store the prepared culture medium at 2-8°C.

The expiration date 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 incubation times as stated below.

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 laboratory 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." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

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 M29: *Protection of Laboratory Workers from Occupationally Acquired Infections*.

Sterilize all biohazard waste before disposal.

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

#### METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

- 1. Suspend 55.0gm of the dehydrated culture media in 1 liter of distilled or deionized water.
- 2. Heat to boiling and mix to dissolve completely.
- 3. Dispense into tubes.

<sup>\*</sup> Adjusted and/or supplemented as required to meet performance criteria.

- 4. Sterilize in the autoclave at 121°C. for 15 minutes.
- 5. Cool in slanted position with deep butts.

## PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed references or refer to the prepared media Instructions for Use (IFU) for Cat. No. L70.

#### LIMITATIONS

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

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results. Always take pH reading at room temperature.

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 autoclaves, incinerators, and incubators, etc., 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 Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	Results
Salmonella enterica ATCC® 14028	С	18-24hr	35°C	Aerobic	Growth; red slant, yellow butt, black butt, H <sub>2</sub> S positive, gas positive
Escherichia coli ATCC <sup>®</sup> 25922	С	18-24hr	35°C	Aerobic	Growth; yellow slant, yellow butt, H <sub>2</sub> S negative, gas positive
Pseudomonas aeruginosa ATCC® 27853	С	18-24hr	35°C	Aerobic	Growth; red slant, red butt, H <sub>2</sub> S negative, gas negative

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

#### **USER QUALITY CONTROL**

Users of dehydrated 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 certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see the reference(s) for more specific information.

#### PHYSICAL APPEARANCE

CRITERION<sup>TM</sup> Kligler Iron Agar (KIA) powder should appear homogeneous, free-flowing, and pinkish-beige in color. The prepared media should appear slightly opalescent with a slight precipitate, and orange-red in color.

### **REFERENCES**

- 1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.
- 2. Tille, P., et al. Bailey and Scott's Diagnostic Microbiology, C.V. Mosby Company, St. Louis, MO.
- 3. Edwards, P.R., and M.A. Fife. 1961. Appl. Microbiol.; 9:478.
- 4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
- 5. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.
- 6. Jorgensen., et al. Manual of Clinical Microbiology, American Society for Microbiology, Washington, D.C.
- 7. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI formerly NCCLS), Wayne, PA.
- 8. J. Med. Res.; 25:217, 1911.
- 9. Am. J. Public Health; 7:1042, 1917.

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