IFU

Instructions for Use

CRITERION™ CAFFEIC ACID AGAR

Cat. no. C7780	CRITERION™ Caffeic Acid Agar	68gm
Cat. no. C7781	CRITERION™ Caffeic Acid Agar	500gm
Cat. no. C7782	CRITERION™ Caffeic Acid Agar	2kg
Cat. no. C7783	CRITERION™ Caffeic Acid Agar	10kg
Cat. no. C7784	CRITERION™ Caffeic Acid Agar	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Caffeic Acid Agar is recommended for the selective isolation and differentiation of *Cryptococcus neoformans*.

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

Cryptococcus neoformans is an encapsulated yeast that produces the enzyme phenoloxidase, an enzyme necessary in melanin synthesis. When in the presence of caffeic acid, the enzyme attacks the acid resulting in the production of melanin. Subsequently, melanin is absorbed by the cell wall of the yeast producing tan to brown pigmented colonies.

The brown pigmented colonies of *Cryptococcus neoformans* were observed by Staib in 1962 when he grew cultures of the yeast on media containing *Guizotia abyssinica* seeds.[6] It was later determined that the seeds contain caffeic acid, which served as the melanin-producing substrate.

In 1966, Shields and Ajello modified Staibs Birdseed Agar by making the medium selective with an antimicrobic additive.[7] CRITERION™ Caffeic Acid Agar is a modification of the latter formula.

FORMULA

Gram weight per liter:	34.0gm/L
Ammonium Sulfate	5.0gm
Glucose	5.0gm
Yeast Extract	2.0gm
Dipotassium Phosphate	0.8gm
Magnesium Sulfate	0.7gm

Caffeic Acid	0.18gm
Chloramphenicol	0.05gm
Ferric Citrate	0.05gm
Agar	20.0gm

Final pH 6.5 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-30ºC. 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 light beige.

Store the prepared culture medium at 2-8ºC.

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 34.0gm of the dehydrated culture media in 1 liter of distilled or deionized water.

2. Heat to boiling and mix to dissolve completely.

3. Sterilize in the autoclave at 121ºC. for 15 minutes.

4. Cool to 45-50ºC.

PROCEDURE

Specimen Collection: Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. Consult listed references for information on specimen collection.[2-5]

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. G213.

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.

Yeasts other than *C. neoformans* may rarely produce brown pigmentation on media containing caffeic acid.

A Sabouraud Dextrose Agar (Cat. no. W70) control should be inoculated in parallel to the Caffeic Acid Agar slant to ensure that a dark pigment is not naturally produced by the colonies. *Aureobasidium*, *Sporothrix*, *Wangiella*, and *Phialophora* may produce dark brown colonies, but the pigment will not be a result of enzymatic activity which is made evident by pigmentation developing in colonies on all media.

Rare strains of *C. neoformans* may not produce pigmented colonies.

Specimens heavily contaminated with bacteria may obscure growth and/or pigmentation of *C. neoformans*.

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	
Cryptococcus neoformans ATCC® 32045	A	72-96hr	15-30°C	Aerobic	Growth; brown to black pigmented colonies
Escherichia coli ATCC® 25922	B	24hr	35°C	Aerobic	Inhibited

* Refer to the document "Inoculation Procedures for Media QC" 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™ Caffeic Acid Agar powder should appear homogeneous, free-flowing, and light beige in color. The prepared media should appear slightly opaque, with precipitate, and light gray 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. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

5. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

6. Staib, F. 1962. *Hyg. Infektionskr. Med. Mikeobiol. Immunol. Virol.*; 148:466-475.

7. Shields, A.B. and Ajello, L. 1966. Medium for Selective Isolation of *Cryptococcus neoformans*, Service; 151:208-209.

8. Denning, D.W., et al. 1990. *Journal of Clinical Microbiology*; Vol. 28, No. 11, p. 2565-2567.

9. La Rocco, Mark, Ph.D. 1992. *Clinical Microbiology Newsletter*; Vol. 14, No. 23, p. 177-181.

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

HDQA 2207B [D]