

Instructions for Use

CAFFEIC ACID DISKS - PHENYLOXIDASE RAPID TEST FOR THE IDENTIFICATION OF *CRYPTOCOCCUS NEOFORMANS*

Cat. no. Z118	Caffeic Acid Disks	25 disks/jar
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INTENDED USE

HardyDisk™ Caffeic Acid Disks are a rapid test to detect the ability of an organism to produce the enzyme phenyloxidase, which is useful for the identification of *Cryptococcus neoformans*.

SUMMARY

Cryptococcosis, specifically caused by *Cryptococcus neoformans*, is a subacute or chronic fungal infection with several manifestations. It is commonly observed as a disseminated disease in the immunocompromised patient with approximately two thirds of patients experiencing meningitis.^(3,5) Because of the wide spectrum of Cryptococcosis and the opportunistic nature of such infection, rapid laboratory identification of *Cryptococcus neoformans* is necessary so that therapy can be immediately initiated. The presumptive identification of *Cryptococcus neoformans* is based on the presence of an encapsulated yeast (using India ink), the absence of pseudohyphae, the failure to utilize an inorganic nitrate substance, and the ability to produce urease. The final identification of *C. neoformans* is usually based on typical substrate utilization patterns and brown pigment production in the presence of caffeic acid.^(2,3,5)

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. Phenyloxidase, an enzyme produced by the organism, reacts with caffeic acid in the presence of iron, resulting in the production of melanin. Subsequently, melanin is absorbed by the cell wall of the yeast producing brown to brown-gray pigmented colonies. As a result, *Cryptococcus neoformans* may be differentiated from other yeasts and from other *Cryptococcus* species by the production of brown pigment in the presence of caffeic acid and ferric citrate.

Hopfer and Groschel developed a rapid six hour pigmentation test in which organisms were inoculated onto caffeic acid and ferric citrate impregnated paper disks.⁽²⁾ In the absence of a positive India ink preparation, *C. neoformans* is not specified until germ tube, chlamydospore, urease and other tests have been performed. Further differentiation using culture media containing caffeic acid can delay the process by three to four days. Through the use of HardyDisk™ Caffeic Acid Disks any suspected colonies can be identified within four hours, therefore decreasing both the time and materials needed to make the identification. The HardyDisk™ Caffeic Acid Disks are a modification of this rapid method and provide for the presumptive identification of *Cryptococcus neoformans* within four hours.

FORMULA

Each HardyDisk™ Caffeic Acid Disk is prepared by impregnating controlled concentrations of caffeic acid and ferric citrate onto a 3/8 inch diameter filter paper disk.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2 to 8°C. away from direct light. Product should not be used if there are any signs of deterioration, discoloration, or if the expiration date has passed. **It is imperative that the product be protected from the light as caffeic acid is light sensitive.** Do not use if disks have any brown, gray, or black discoloration. In addition, protect product from excessive heat and moisture.

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 Precautions for blood. 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." 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.

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.

Method of Use:

1. Prior to use, allow the disks to equilibrate to room temperature.
2. Place the disk on a slide with one drop of sterile water and place into an empty sterile petri plate. Add a moist piece of gauze to the petri plate to prevent the disk from drying out during incubation. Alternatively, the disk can be placed on the agar surface of a non-dextrose containing medium, such as Corn Meal Agar (Cat. no. W17), for rehydration. Refer to note in the "Limitations" section below.
3. Inoculate the disk with several (five to six colonies) yeast colonies from a 48-72 hour old culture to yield a visible cell paste on the disk surface.
4. Replace the plate lid and incubate the disks aerobically at 35°C. in the dark.
5. Observe for the development of dark brown pigmentation at 30 minute intervals for up to four hours. No color development within four hours indicates a negative result.

INTERPRETATION OF RESULTS

Development of dark brown to brown-gray color on the disk surface within four hours is a positive result indicative of phenyloxidase activity found in *Cryptococcus neoformans*. Very light non-specific pigmentation may be produced by *Cryptococcus albidus* and *Cryptococcus laurentii*. The intensity of this non-specific reaction will remain the same even after 24 hours of incubation.

LIMITATIONS

The addition of more than one drop of sterile water to the disk will delay the results or may give false-negative results.

This product is used in conjunction with other tests to identify cultures of *C. neoformans*. It is necessary to confirm, by other biochemical tests, the identification of all organisms suspected of being *C. neoformans*.^(2,3,9)

With occasional isolates (particularly serotype C), the production of phenyloxidase may have to be induced. Rare strains of *C. neoformans* may not produce a positive reaction.^(2,9)

A blank control disk (Cat. no. Z7121) can be inoculated in parallel to the HardyDisk™ Caffeic Acid Disk to ensure that a dark pigment is not naturally produced by the colonies. *Aureobasidium*, *Sporothrix*, *Wangiella* and *Phialophora* may produce dark brown colonies. However the pigment will not be a result of enzymatic activity, which is made evident by pigmentation developing in colonies on both the HardyDisk™ Caffeic Acid Disk and the control disk.^(2,9)

Yeasts other than *C. neoformans*, such as *C. albidus* and *C. laurentii*, may produce light brown non-specific pigmentation in the presence of caffeic acid.⁽⁹⁾

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, slides, petri plates, 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 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	
<i>Cryptococcus neoformans</i> ATCC® 32045	E	1-4hr	35°C	Aerobic	Dark brown to brown-gray color
<i>Cryptococcus albidus</i> ATCC® 34140	E	4-24hr	35°C	Aerobic	Very light tan; non-specific reaction
<i>Candida albicans</i> ATCC® 10231	E	4hr	35°C	Aerobic	No color change

* 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 certificate of analysis (CofA) available from Hardy Diagnostics [Certificate of Analysis](#) website. Also refer to the document "[Finished Product Quality Control Procedures](#)," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

HardyDisk™ Caffeic Acid Disks are 3/8 inch filter paper disks and should appear white to very light off-white in color.



Cryptococcus neoformans (ATCC® 34877) positive reaction on Caffeic Acid Disks (Z118). Incubated aerobically for four hours at 35°C.



Cryptococcus albidus (ATCC® 34140) non-specific reaction on Caffeic Acid Disks (Z118). Incubated aerobically for four hours at 35°C.



Candida albicans (ATCC® 10231) negative reaction on Caffeic Acid Disks (Z118). Incubated aerobically for four hours at 35°C.

REFERENCES

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IFU-10311[A]



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[Ordering Information](#)

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