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TECHNICAL DATA SHEET 316

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Fungi-Fluor[®] Kit

for the Fluorescence Detection of Fungal and Bacterial Elements

INTRODUCTION

The Fungi-Fluor[®] Kit can be used for the rapid identification of various fungal infections in fresh or frozen clinical specimens, paraffin or glycol methacrylate embedded tissues.^{1, 2} Typical specimens include sputum, bronchoalveolar lavage (BAL), bronchial wash, and tissue biopsies.

Fungi-Fluor[®] Staining Solution is a 0.05% solution of high purity Cellufluor in deionized water with potassium hydroxide added as a clearing agent. The Fungi-Fluor[®] Counterstaining Solution B is an aqueous solution of Evans Blue dye used to reduce background fluorescence. Cellufluor binds nonspecifically to beta-linked polysaccharides found in the cell walls of various organisms such as chitin and cellulose.^{2, 3}

Various fungal and yeast types will stain fluorescently including *Candida* sp., *Histoplasma* sp. and *Aspergillus* sp. among others.^{1, 6} The kit will also stain *Pneumocystis carinii* cysts,^{1, 7} parasites such as *Plasmodium* sp., and regions of fungal hyphae undergoing differentiation.⁶⁻⁹ Keratin, collagen, and elastin fibers are also stained and may provide structural guidelines for diagnosis.

ADVANTAGES/BENEFITS OF FUNGI-FLUOR[®] KIT

- Accurate diagnosis - more accurate than KOH preps.
- Faster diagnosis - more rapid than PAS (Periodic Acid-Schiff) or GMS (Gomori Methenamine Silver) stains.
- Will not interfere with subsequent PAS or GMS stains if used for confirmation sealed with Poly-Mount (*Cat. #08381*) Coverslipping Media after dehydration.
- Restain stored specimens to enhance time diminished fluorescence.
- Counterstain minimizes background fluorescence and aids in diagnosis.
- Packaged in convenient dropper bottles.

SPECIMEN PREPARATION

The specimen or smear should be fixed in absolute methanol for 1 to 5 minutes. This may vary with the thickness of the smear. The fixation step will help adhere the specimen to the slide and prevent loss of material during staining. Frozen sections or specimens should be fixed in absolute methanol for 5 to 10 minutes depending on section thickness or laboratory protocol. Slides can be stained immediately after rinsing with distilled or deionized water.

Microbial Genera and Tissue Elements That are Stained by Calcofluor White

Fungi	Degree of Fluorescence*
<i>Sporothrix schenckii</i>	++++
<i>Scopulariopsis brevicaules</i>	++++
<i>Rhizopus oryzae</i>	++++
<i>Penicillium thomii</i>	++++
<i>Fusarium oxysporum</i>	++++
<i>Aspergillus fumigatus</i>	++++
<i>Trichophyton mentagrophytes</i>	++++
<i>Torulopsis glabrata</i>	++++
<i>Sporobolomyces salmonicolor</i>	++++
<i>Saccharomyces cerevisiae</i>	++++
<i>Cryptococcus neoformans</i>	++++
Bacteria	
<i>Nocardia asteroides</i>	+
<i>Streptomyces griseus</i>	+
<i>Actinomyces israelii</i>	+
<i>Mycobacterium gordonae</i>	+
<i>Escherichia coli</i>	+
<i>Klebsiella pneumoniae</i>	+
<i>Staphylococcus aureus</i>	+
<i>Staphylococcus epidermidis</i>	+
<i>Group B Streptococcus</i>	+
Tissue Elements	
<i>Collagen</i>	++++
<i>Elastin</i>	++++
<i>Keratin</i>	++++

*Relative degrees of fluorescence expressed as
+ (least) to ++++ (greatest)

(Adapted from Arch. Pathol. Lab. Med., Vol 108, p. 618, 1984)

STAINING PROCEDURE

1. Place slide in a horizontal position and apply a few drops of Fungi-Fluor® Solution A directly to the specimen. The entire specimen should be covered or flooded. Stain for 1 minute.
2. Drain the solution off the slide and rinse very gently with distilled or deionized water. Slides can be gently dipped in two changes of distilled or deionized water.
3. Place the slide in a horizontal position and apply a few drops of Fungi-Fluor® Solution B Counterstain directly to the specimen. The entire specimen should be covered or flooded. Stain for 1 minute.
4. Drain the solution off the slide and rinse very gently with running distilled or deionized water. Slides can be gently dipped in two changes of distilled water.
5. Slides can now be dehydrated quickly through 2 changes each of 95% ethanol, absolute ethanol, and then xylene followed by coverslipping with Poly-Mount® (Cat. #08381) Coverslipping Media.

Note: Cell cultures or scrapings can be stained directly by adding 1 or 2 drops of Fungi-Fluor® Solution A and Solution B to the preparation and viewing directly or with a coverslip.

MICROSCOPE FILTER REQUIREMENTS

Fluorescence microscope filter descriptions can vary with the type of microscope used. We suggest you discuss filter options with your specific microscope manufacturer to assure proper excitation levels for viewing the specimen. The microscope manufacturer should have a list of filters with common descriptions to match your needs.

1. Ultra Violet, Calcofluor White Filter - Excitation 340nm to 380nm with Suppression Filter 430nm. The color of the fluorescent material should be blue with a red background if counterstain is used in the procedure.
2. H3 Violet Plus Blue, Wide Band FITC Filter - Excitation 420nm to 490nm with Suppression Filter 515nm. The color of the fluorescent material should be green with a red background if the counterstain is used in the procedure.

The filters listed all give yellow-green or apple-green fluorescence.

- Leica/Lietz D, G, or H3 filters
- Olympus Blue Exciter Cube
- Nikon B-3A Filter Cube
- Riechert-Jung 713
- Ziess 05, 06, and 07 Filter Cubes

LIMITATION OF PROCEDURE

The Fungi-Fluor® Kit is intended for use as a non-specific fluorescent stain for various fungal types and elements. The kit cannot be used to identify specific organisms on the basis of fluorescence alone. Morphological staining features must be used in conjunction with other laboratory data to identify a particular organism.

PRECAUTIONS AND STORAGE

Fungi-Fluor® Solution is classified as an irritant. Exercise normal care in handling. Fungi-Fluor® Solutions should be stored protected from light at room temperature. Do not freeze.

REFERENCES

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3. J. Clin. Microbiol., 29(3):645 (1991).
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6. Methods in Microbiol., Vol. 5A, Academic Press, NY, pp. 135-144 (1971).
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9. Stain Technol., 60(2):69 (1958).

ORDERING INFORMATION

Cat. #	Description	Size
17442	Fungi-Fluor® Kit	1 kit

ADDITIONAL PRODUCT

08381	Poly-Mount® Coverslipping Media	120ml 940ml
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TO ORDER

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