CALCOFLUOR WHITE STAIN KIT

INTENDED USE
Remel Calcofluor White Stain Kit is recommended for use in qualitative procedures as a rapid, non-specific fluorochrome stain for the initial microscopic detection of fungal elements, yeasts, Acanthamoeba cysts, microsporidia, and Pneumocystis carinii in clinical specimens.

SUMMARY AND EXPLANATION
In 1961, Darken reported the uptake of a fluorescent brightener, calcofluor white, by actively growing cultures of yeasts and higher fungi.1 Hageage and Harrington described the use of calcofluor white (CFW) to demonstrate hyphae and yeasts in clinical specimens.2 Monheit et al. applied this stain to frozen sections of lung and soft tissues for the intraoperative diagnosis of fungal infection.3 More recently calcofluor white has been used in the detection of fungi in direct preparations and in deparaffinized tissue sections.4,5 Basetski and Robison reported the use of calcofluor white in the detection of P. carinii cysts in bronchoalveolar lavage (BAL) specimens.6 Milligan confirmed these findings in 1992, reporting BAL samples useful for detection of infections caused by other opportunistic fungi, as well as P. carinii in immunocompromised patients.7 Wilhelmus et al. demonstrated the fluorescence of Acanthamoeba keratitis in corneal scrapings by the CFW stain.8 Weber et al. reported that chemofluorescent optical brightening agents, such as calcofluor white, also stain microsporidia spores.9

PRINCIPLE
Calcofluor white is a non-specific fluorochrome stain that binds with cellulose and chitin. Upon excitation with long wave ultraviolet light, this compound delineates the cell walls of cellulose-containing organisms.2 Prior to staining with calcofluor white a clearing agent, such as potassium hydroxide, is added to the specimen to dissolve tissue cells. Evans blue dye is incorporated in the stain to minimize background material.

REAGENTS (CLASSICAL FORMULAE)*
Reagent A:
- Potassium Hydroxide (CAS 1310-58-3) ......................125.0 g
- Glycerin (CAS 56-81-5) ........................................125.0 g
- Demineralized Water (CAS 7732-18-5).........................1000.0 ml

Reagent B:
- Calcofluor White (CAS 4404-43-7) ..................1.0 g
- Evans Blue Dye (CAS 314-13-6) ..........................0.4 g
- Demineralized Water (CAS 7732-18-5).........................1000.0 ml

*Adjusted as required to meet performance standards.

PRECAUTIONS
DANGER! POISON, may be harmful or fatal if swallowed. CORROSIVE, may cause burns or irritation to skin, eyes and respiratory tract. Avoid breathing vapor and eye/skin contact.

This product is for In Vitro diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully. Refer to Material Safety Data Sheet for additional information.

MATERIALS SUPPLIED
- Potassium Hydroxide (Reagent A)
- Calcofluor White and Evans Blue Dye (Reagent B)
- Instructions For Use (IFU)

STORAGE
This product is ready for use and no further preparation is necessary. Store product in its original container at room temperature until used. Protect product from light.

PRODUCT DETERIORATION
This product should not be used if (1) the color has changed, (2) the expiration date has passed, or (3) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT
Specimens should be collected and handled following recommended guidelines.10,11

MATERIALS REQUIRED BUT NOT SUPPLIED

PROCEDURE
Consult fluorescent microscope manufacturer instructions or appropriate references for filter recommendations suitable for use with CFW stain preparations.10,12

Fungal elements and yeasts:
1. Place the specimen on a clean glass slide.
2. Add 1 drop of Reagent A and gently mix.
3. Add 1 drop of Reagent B and mix.
4. Cover with a clean glass coverslip and examine the slide using a fluorescent microscope. Observe for fluorescence and typical morphology.

Acanthamoeba cysts:
1. Place the specimen on a clean glass slide and allow to air dry. Appropriate specimens include: corneal scrapings or biopsy, conjunctiva or corneal ulcer, contact lens paraphimia, and concentrated water samples of at least 100 ml. Other specimens may be appropriate if the infection is disseminated.
2. Fix slide in methanol for 2 minutes.
3. Paraffinized sections (6 µm thick) may also be used.
   a. Soak slide in a xylene substitute for 1-2 minutes to remove paraffin.
   b. Dip slide in 100% alcohol twelve times.
   c. Dip slide in 95% ethanol twelve times.
   d. Rinse gently in demineralized water and drain the slide.
4. Add 1 drop of Reagent A and gently mix.
5. Add 1 drop of Reagent B and mix.
6. Stain for five (5) minutes. Remove excess stain by rinsing in demineralized water. Cover slide with a clean glass coverslip and examine the specimen using a fluorescent microscope. Observe for fluorescence and typical morphology.

Microsporidia:
1. Although the most common specimen is a fresh or preserved (formalin or SAF) stool specimen, other specimens such as tissues, duodenal aspirates, concentrated urine, sputum, CSF, nasal discharge, BAL, and conjunctiva are also appropriate. Place a thin specimen (10 µl) on a clean glass slide and heat-fix on a slide warmer at 60°C until dry.
2. Fix slide in methanol for 2 minutes.
3. Add 1 drop of Reagent A and gently mix.
4. Add 1 drop of Reagent B and mix.
5. Stain for one minute and remove excess stain by rinsing in demineralized water. Cover slide with a clean glass coverslip and examine the specimen using a fluorescent microscope. Observe for fluorescence and typical morphology.

Fungal elements and yeasts:
1. Place the specimen on a clean glass slide.
2. Add 1 drop of Reagent A and gently mix.
3. Add 1 drop of Reagent B and mix.
4. Cover with a clean glass coverslip and examine the slide using a fluorescent microscope. Observe for fluorescence and typical morphology.

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**Pneumocystis carinii:**
1. The specimens of choice are concentrated BAL (10-25 μl) or tissue samples. Decreased sensitivity is observed with induced sputum. Place specimen on a clean glass slide and to air dry.
2. Fix slide in methanol for 2 minutes.
3. Add 1 drop of Reagent A and gently mix.
4. Add 1 drop of Reagent B and mix.
5. Stain for one (1) minute. Remove excess stain by rinsing in demineralized water. Cover slide with a clean glass coverslip and examine the specimen using a fluorescent microscope. Observe for fluorescence and typical morphology.

**INTERPRETATION**
- **Fungal elements, yeasts:** Bright apple-green fluorescence with typical morphology.
- **Acanthamoeba cysts:** Cysts (10-25 μm) appear corrugated and double walled with bright apple-green fluorescence and bright orange cytoplasm.
- **Microsporidia:** Apple-green fluorescence; intestinal microsporidia spores range in size from 0.9-1.5 μm to 1.2-2.0 μm, cell wall is brightened but staining is not specific.
- **Pneumocystis carinii:** Brilliant apple green fluorescence, cysts are 5-8 μm in diameter and contain up to 8 crescent or pleomorphic shaped sporozoites; cell wall and double parenthesis structures inside the cysts stain intensely.
- **Bacteria:** Weak to no fluorescence; typical coccoid or bacillary shape.

**QUALITY CONTROL**
All lot numbers of Calcofluor White Stain Kit have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

**CONTROL**
- **Trichophyton mentagrophytes** ATCC® 9533: Bright green fluorescence
- **Escherichia coli** ATCC® 25922: Weak to no fluorescence

**LIMITATIONS**
1. **CFW** is a fluorescent brightener that aids in the detection of certain microorganisms by means of morphological delineation. Definitive identification may require additional biochemical and serological testing, or confirmation by an alternate staining technique.
2. Studies indicate that the capsule of Cryptococcus will not stain with CFW. Alternate techniques, such as direct examination using India Ink, are recommended for the detection of this organism.
3. Bacteria and debris may fluoresce, but less brightly than fungi.
4. Both Reagent A and B must be added for P. carinii, Acanthamoeba, and microsporidia to adequately fluoresce.
5. Brightener-induced fluorescence fades with prolonged viewing, especially in thinner sections, but fluorescence may be restored by restaining.
6. If a specimen contains excessive, nonspecific debris or yeast, further examination for microsporidia should be performed by another staining method. All positives for microsporidia should be confirmed by the Modified Trichrome stain.

**BIBLIOGRAPHY**

**PACKAGING**
REF R40015, Calcofluor White Stain Kit …………... 2 Btl/Kit, 50 ml/Btl

**Symbol Legend**
- **REF** Catalog Number
- **IVD** In Vitro Diagnostic Medical Device
- **LAB** For Laboratory Use
- **(IFU)** Consult Instructions for Use (IFU)
- **LOT** Batch Code (Lot Number)
- **Use By (Expiration Date)**

ATCC® is a registered trademark of American Type Culture Collection.
CAS (Chemical Abstracts Service Registry No.)

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