



# CALCOFLUOR WHITE STAIN

- For in vitro use only -

Catalogue No. SC15

Our Calcofluor White Stain can be used as a fluorescent stain for yeasts, fungi and parasitic organisms.

Calcofluor White is a non-specific fluorochrome that binds with cellulose and chitin contained in the cell walls of fungi and other cellulose-containing organisms. Staining specimens with Calcofluor White Stain is a rapid procedure and has been described as a rapid method for the detection of many yeasts and pathogenic fungi, as well as *Pneumocystis carinii*, *Microsporidium*, *Acanthamoeba*, *Naegleria*, and *Balamuthia* species. Calcofluor White Stain can be mixed with a potassium hydroxide mixture to clear up the specimen to facilitate visualization of fungal elements.

Evans blue is added to act as a counterstain and diminishes background fluorescence of tissues and cells when using blue light excitation (not UV). Other biological materials fluoresce reddish-orange versus the bright apple-green of stained fungal or parasitic elements.

## Formulation per Litre

Calcofluor White M2R.....1.0 g  
Evans Blue .....0.5 g

## Recommended Procedure

1. Put the specimen to be examined onto a clean glass slide.
2. Add one drop of Calcofluor White Stain and one drop of 10% Potassium Hydroxide (Dalynn RP85).
3. Place a coverslip over the specimen. Let the specimen stand and stain for 1 minute.
4. Cover the slide with a paper towel and gently press to remove any excess fluid.
5. Examine the slide under UV light at x100 to x400 magnification.

## Interpretation of Results

Calcofluor white aqueous solutions show absorption over the range of 300 to 412-nm, with an absorbance peak at 347-nm. This means that maximum excitation and fluorescence occurs with ultraviolet light, although excitation with violet or blue violet light also gives good results.

Fungi, *Pneumocystis* cysts, and parasites stained with calcofluor white display a brilliant apple-green fluorescence under ultraviolet, violet, and blue light. The green coloration is due to the barrier filters commonly used in fluorescence microscopy. Care must be taken when interpreting calcofluor white staining because of non-specific reactions that may occur. Yellowish-green background fluorescence may be observed with tissue samples, although the fluorescence displayed by fungal or parasitic structures is much more intense and highly visible. Background fluorescence can be diminished by examining slides under blue light or by using various exciter and barrier filter combinations.

*Pneumocystis* cysts are normally 5 to 8  $\mu\text{m}$  in diameter, round, uniform in size, and exhibit a characteristic peripheral cyst wall staining with an intense internal “double-parenthesis-like” structure. Yeast cells can be differentiated from *P. carinii* by budding and deep internal staining.

- *Cotton fibers will fluoresce strongly and must be differentiated from fungal hyphae*
- *Background fluorescence maybe present but fungi exhibit a more intense fluorescence.*
- *Amebic cysts fluoresce but trophozites will not stain or fluoresce*

## Quality Control

| Organism                              | Expected Result |
|---------------------------------------|-----------------|
| <i>Candida albicans</i><br>ATCC 10231 | Fluorescence    |



Fig 1. Microsporidian spores in urine

## Storage and Shelf life

Our Calcofluor White Stain should be stored at room temperature and protected from light. Under these conditions it has a shelf life of 52 weeks from the date of manufacture.

## References

1. Hageage GJ, Harrington BJ. Use of calcofluor white in clinical mycology. Lab Med 1984; 15:109-12.
2. Wilhelmus KR, Osato MS, Font RL, Robinson NM, Jones DB. Rapid diagnosis of *Acanthamoeba keratitis* using calcofluor white. Arch Ophthalmol 1986; 104:1309-12.
3. Green LK, Cowan DF, Moore DO. Examination of tissue for parasites by fluorescent microscopy. Histopathology 1988; 12:553-5.
4. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's diagnostic microbiology. 10th ed. St. Louis: Mosby, 1998.
5. Murray PR, Baron E, Pfaller M, Tenover F, Tenover FC. Manual of clinical microbiology. 7th ed. Washington: ASM, 1999.
6. Harrington BJ, Hageage GJ. Calcofluor white: a review of its uses and applications in clinical mycology and parasitology. Lab Med 2003; 34:361-7.

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