Technical Bulletin

Calcofluor White Stain

Suitable for microbiology

Catalog Number 18909

Product Description

Calcofluor White Stain is a non-specific fluorochrome that binds with cellulose and chitin contained in the cell walls of fungi and other organisms. The staining procedure using Calcofluor White Stain is a rapid method for the detection of many yeasts and pathogenic fungi such as *Microsporidium*, *Acanthamoeba*, *Pneumocystis*, *Naegleria*, and *Balamuthia* species.

Calcofluor White Stain is provided as an aqueous solution with the following composition:

Reagent	Concentration
Calcofluor White M2R	1 g/L
Evans Blue	0.5 g/L

Evans blue acts as a counterstain and diminishes the background fluorescence of tissues and cells when using blue light excitation (not UV). A range of 300 nm - 440 nm ($\lambda_{Em\ max}$ 433 nm; 0.1 M phosphate pH 7.0 with cellulose) is possible for emission wavelength (λ_{Em}) with excitation (λ_{Ex}) set at approximately 355 nm.

One drop of 10% potassium hydroxide solution can be added to the sample slide for better visualization of fungal elements.

Precautions and Disclaimer

For R&D Use Only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the Calcofluor White Stain at room temperature and protected from light.

Procedure

1. Put the sample to be examined onto a clean glass slide.
2. Add one drop of Calcofluor White Stain and one drop of 10% Potassium Hydroxide solution (not included)
3. Place a coverslip over the specimen and let stand for 1 minute.
4. Examine the slide under UV light (λ_{Ex} = 355 nm) at 100× to 400× magnification.

Results

Fungal or parasitic organisms appear fluorescent bright green to blue, while other material is fluorescent reddish orange.

Non-specific reactions may occur when tissue samples are used. A yellowish-green background fluorescence may be observed with tissue specimens, but fungal and parasitic organisms will have a more intense fluorescence.

Note: Cotton fibers fluoresce strongly and must therefore be differentiated from fungal hyphae. In addition, amoebic cysts will fluoresce when treated with Calcofluor White Stain, but trophozoites will not stain or fluoresce.

Background fluorescence may be reduced by using blue light or by using different combinations of emission and excitation filters.

References

1. Monheit, J.E., et al., Rapid detection of fungi in tissues using calcofluor white and fluorescence microscopy, *Arch. Pathol. Lab. Med.,* **108**, 616-618 (1984).
2. Hageage, G.J., and Harrington, B.J., Use of calcofluor white in clinical mycology, *Lab. Med*., **15**, 109-12 (1984).
3. Wilhelmus, K.R., et al., Rapid diagnosis of Acanthamoeba keratitis using calcofluor white, *Arch. Ophthalmol.*, **104**, 1309-12. (1986).
4. Green, L.K., et al., Examination of tissue for parasites by fluorescent microscopy. *Histopathology*, **12**, 553-5 (1988).
5. Harrington, B.J., and Hageage, G.J., Calcofluor white: tips for improving its use, *Clin. Microbiol. Newsl*., **13**, 3-5 (1991).
6. Mason, D.J., et al., The ability of membrane potential dyes and calcofluor white to distinguish between viable and non-viable bacteria, J. *Appl. Bacteriol.*, **78**, 309-315 (1995).
7. Forbes, B.A., et al., Bailey and Scott's diagnostic microbiology, 10th ed. St. Louis, Mosby, (1998).
8. Murray, P.R., et al., Manual of clinical microbiology, 7th ed. Washington, ASM, (1999).
9. Harrington, B.J, and Hageage, G.J., Calcofluor white: a review of its uses and applications in clinical mycology and parasitology, *Lab. Med*., **34**, 361-7 (2003).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Technical Service

Visit the tech service page on our web site at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.