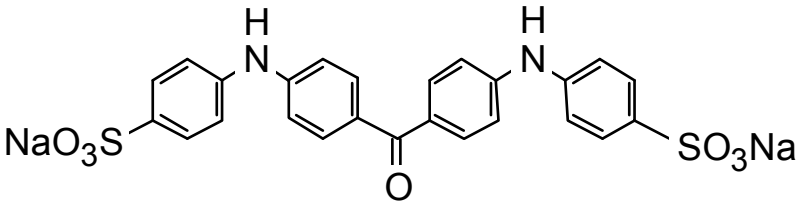
ANILINE BLUE FLUOROCHROME

FOR RESEARCH PURPOSES ONLY

Cat. No. 100-1



APPLICATIONS

Plant histochemistry:
The synthetic fluorochrome can be used in place of decolourized aniline blue to detect callose deposits in plants (Jensen, 1962; Eschrich & Currier, 1964). Callose deposits contain (1→3)-β-glucan as the essential component; they appear during tissue development, in mechanical and temperature stress and in the response of plant tissues to pathogenic and symbiotic infections. Staining with decolourized aniline blue has two disadvantages that are overcome by using the fluorochrome.

- Solutions of crude aniline blue are buffered to pH above 8.5 to maintain the dye (an acid-base indicator) in its colourless form.
- There is significant background fluorescence in sections of plant tissues due to complexing of the fluorochrome with polysaccharides other than the β-glucans in callose deposits.

The fluorochrome

- can be used at any pH between 3 and 12 (Evans *et al*., 1983).
- is compatible with a wide range of counter stains.

At low ionic strength, the fluorescence with cellulose is very low, while that of insoluble (1→3)-β-glucans such as pachyman and paramylon is unchanged. There is dramatic improvement in contrast of staining of callose deposits in tissue sections with fluorochrome solutions in water compared with decolourized aniline blue (Clarke *et al*, 1983).

Analytical probe for (1→3)-β-glucans:
The specificity of fluorochrome for complexing with (1→3)-β-glucans is high (Evans *et al*., 1983). It can be used for the fluorimetric determination of (1→3)-β-glucans (Kauss, 1989), in the quantitation of (1→3)-β-glucan synthase products and in the determination of (1→3)-β-glucan hydrolase activity based on loss of fluorescence of substrate molecules after depolymerization.

Inhibitor of (1→3)-β-glucan synthase (EC2.4.1.34):
The fluorochrome is a potent inhibitor of (1→3)-β-glucan synthase in petiole tissue of sugar beet (Morrow and Lucas, 1986).

CHEMICAL PROPERTIES

Aniline blue fluorochrome is a chemically synthesized fluorochrome identical with the component of commercial aniline blue (CI 42755) which reacts with (1→3)-β-glucans to give a brilliant yellow fluorescence in UV light.

The fluorochrome, 4'4-[carbonyl bis (benzene 4,1-diyl) bis (imino)] bis benzensulphonic acid: $C_{23}H_{18}N_2Na_2O_7S_2O_2$ (sodium salt) mol. wt. 586, is a crystalline, water-soluble, solid, m.p. >300^{o}C (Evans & Hoyne 1982). The aqueous solution is non-fluorescent, in alcoholic solutions the fluorescence increases with decreasing polarity of the alcohol. (In water, λ_{ex} 390nm, λ_{em} 480nm; in butan-1-ol, λ_{ex} 380nm, λ_{em} 470mm). The relative intensity of fluorescence in butan-1-ol is 150 times that in water.

METHOD OF USE

Prepare a stock solution of 0.1mg/mL in distilled water. This stock can be stored at 4^{o}C in the dark for at least one year. Dilute the stock solution 1:3 with either water or desired buffer (e.g. 0.1M K_3PO_4, pH12.0) before use.

Incubate tissue sections (fresh or embedded) with fluorochrome solutions (20μL/section) for 30 min at 20^{o}C, wash in water or buffer and examine by fluorescence microscopy.

At this rate of use 1mg will give 10mL of stock solution and 40mL of working solution. Using 20μL/section, 2000 sections can be stained from one 1mg unit of fluorochrome.

REFERENCES

Eschrich, W. & Currier, H.B. (1964). *Stain Technology 39:* 303.

Evans, N.A. & Hoyne, P.A. (1982). *Aust. J. Chem. 35:* 2571.

Evans, N.A., Hoyne, P.A. & Stone, B.A. (1984) *Carbohydrate Polymers 4:* 215-230.

Jensen, W.A. (1962) *Botanical Histochemistry*, W.H. Freeman & Co., San Francisco.

Kauss, M. (1989). *Modern Methods of Pant Anal. New Series 10:* 127-137.

Morrow, D.L. & Lucas, W.J. (1986) *Plant Physiol. 81:* 171-176.

Schedletzky, E., Unger, C. & Delmer, D.P. (1997) *Anal. Biochem.* 249: 88-93.

Stone, B.A., Evans, N.A., Bonig, I & Clarke, A.E. (1984) *Protoplasma 122:* 191-195.

Biosupplies Australia Pty Ltd

A.B.N. 62 006 209 034

P.O. Box 187, La Trobe Uni, Bundoora, Victoria 3083, AUSTRALIA

www.biosupplies.com.au