INSTRUCTIONS

Fluoraldehyde™ *o*-Phthalaldehyde Crystals

26015

0663.2

Number	Description
26015	**Fluoraldehyde *o*-Phthalaldehyde Crystals**, 5g
	Molecular Weight: 134.12
	Detection: Fluorescence (λ_{ex} = 340nm, λ_{em} = 455nm)

Storage: Upon receipt store at room temperature protected from moisture and air.

Introduction

The Thermo Scientific Fluoraldehyde *o*-Phthalaldehyde (OPA) Crystals are a specially purified fluorogenic grade of *o*-phthalaldehyde. They are intended for high sensitivity detection of primary amines, including amino acids, peptides, proteins and polyamines in pre- and post-column chromatographic effluents. First described by Roth[1,2] for detection of amino acids, the reagent solution formulation was later modified by Benson and Hare[3] for detection of peptides. The solution formulation described in this instruction booklet is a further modification.

Important Product Information

- Fluoraldehyde OPA Crystals and 2-mercaptoethanol are subject to atmospheric oxidation, and these oxidation products can contribute to increased background fluorescence. Therefore, unless the reagent solution is protected from atmospheric oxygen, it should be prepared just before use. The solution may be stored under nitrogen in closed glass bottles at 4°C for up to two weeks without increased fluorescence.
- The reagent solution described by Roth[2] contained 800mg of *o*-phthalaldehyde per liter. Because Fluoraldehyde OPA Crystals are of much greater purity than that from other sources, reagent solutions containing 500mg Fluoraldehyde OPA Crystals per liter have been used successfully. Even lower concentrations may be suitable for some applications, but linearity of fluorescent response should be confirmed.
- Fluorescence response with amino acids increases as the pH of the Fluoraldehyde OPA reagent solution is increased, except with histidine, which shows a decrease in fluorescence. Any reagent solution pH value between 9.0 and 11.5 will yield effective fluorescence. Above pH 11.5, the solution becomes unstable. It is important to carefully control pH of each batch of reagent solution to ensure that the relative fluorescence of each assayed substance remains constant.

Additional Materials Required

Note: Use reagent grade materials.

- Boric acid
- 2-Mercaptoethanol (Product No. 35602)
- Potassium hydroxide, 45% solution
- Brij®-35 Detergent, 30% solution (Product No. 20150)
- Ultrapure water, preferably with resistance of 16megohm cm^{-1}
- Methanol

Preparation of OPA Reagent Solution for Detection of Amino Acids and Peptides

- With stirring, dissolve 25g of boric acid in 950mL of ultrapure, deaerated water.
- Use a pH meter to monitor the pH and titrate with the potassium hydroxide solution (approximately 30mL will be required) to a final pH of 10.40 ± 0.02.
- Add 3.0mL of 30% Brij-35 Detergent solution.
- Add 2.0mL of 2-mercaptoethanol.
- Dissolve 500-800mg of Fluoraldehyde OPA Crystals (see Important Product Information Section) in approximately 10mL of methanol (or 95% ethanol) at room temperature using gentle swirling.
- Add the methanol-OPA solution to the boric acid buffer. Final volume is ~1000mL.

  Note: For post-column analysis, Fluoraldehyde OPA Reagent Solution flow rate should equal that of the column effluent.
- Fluoraldehyde OPA Reagent Solution has excitation maxima of 340nm (excitation range 330-390nm) and emission maxima of 455nm (emission range 436-475nm).

Related Thermo Scientific Products

26025 **Fluoraldehyde Reagent Solution,** 120mL

References

1. Roth, M. (1971). Fluorescence reaction for amino acids. *Anal Chem* **43:**880-2.
2. Roth, M. and Hampai, A. (1973). Column chromatography of amino acids with fluorescent detection. *J Chromatogr* **83:**353-6.
3. Benson, J.R. and Hare, P.E. (1975). o-phthalaldehyde: Fluorogenic detection of primary amines in the picomole range. Comparison with fluorescamine and ninhydrin. *Proc Nat Acad Sci USA* **73:**619-22.
4. Weidekamm, E., Wallach, D. and Fluckiger, R. (1973). A new sensitive rapid fluorescence technique for the determination of proteins in gel electrophoresis and in solution. *Anal Biochem* **54:**102.