



CATALYST FOR SUCCESS

## ➔ O-PHTHALALDEHYDE REAGENT

FOR AUTOMATED POST-COLUMN  
DERIVATIZATION OF PRIMARY AMINES

### INTRODUCTION

Primary amines form highly fluorescent adducts when reacted with *o*-Phthalaldehyde (OPA) and a mercaptan under basic conditions. At a pH>9 and ambient temperature, reaction is generally complete within 30 seconds. The products of this reaction, 1-alkyl-2-alkylthio-substituted isoindoles, exhibit optimal excitation at 330 nm and maximal emission at 465 nm. The reagents described below are intended for postcolumn derivatization of primary amines such as:

- amino acids in native samples and protein or tissue digests
- those formed by post-column cleavage of carbamate insecticides or glyphosate herbicide
- those derived from other sources.

### REAGENT COMPONENTS

- Pour into your reservoir and use; the ultimate in convenience with a minimum of handling.
- Three-month shelf life.

### *o*-Phthalaldehyde, Chromatographic Grade™

Pickering's OPA is specially prepared to meet the demanding requirements of highsensitivity pre- and post-column HPLC derivatization methods. Our repurification process actually begins with commercially available 99 % material.

The entire process is controlled to prevent trace amine contamination. Lot quality is verified by post-column HPLC using a high-sensitivity fluorescence detector.

### Thiofluor™, Chromatographic Grade™

Pickering's Thiofluor, a solid, nearly odorless nucleophile, is a superior substitute for 2-Mercaptoethanol in the preparation of OPA reagents. It forms a more stable reagent and longer-lasting fluorophore with OPA than does 2-Mercaptoethanol, yet it has the same fluorescence properties.

Unlike the volatile 2-Mercaptoethanol, Thiofluor will not migrate through the gas manifold and regulator of the OPA reagent pressurization system.

Two grams of Thiofluor is equivalent to 1 mL of 2-Mercaptoethanol.



***o*-Phthalaldehyde Diluents, Chromatographic Grade™**

Three application-specific diluents are available from Pickering Laboratories: CB910 and GA104 for carbamate and glyphosate analyses, and OD104 for amino acid analysis.

These borate solutions are produced from starting materials which are free of heavy metals and amines. As with most other products of Pickering Laboratories, the quality of the OPA Diluents is verified by actual post-column HPLC analysis.

**REAGENT PREPARATION*****Storage and Handling***

The prepared reagent is sensitive to oxygen. It should be formulated in a container which can serve as the instrument reservoir, such as the 1L reservoir assembly included with the Pickering post-column instrument. Always store, transfer, and use the prepared reagent under an inert gas (N<sub>2</sub>). Stored under inert gas in the glass reagent reservoir, the reagent will maintain its original signal-to-noise ratio for one week or more.

***OPA Reagent for Carbamate Insecticide Analysis***

- 1 Pour 945 mL of *o*-Phthalaldehyde Diluent (Cat. No. CB910) into the reservoir. Save 5 mL for Step 5.
- 2 Thoroughly de-aerate the contents by sparging with the same inert gas used to protect the reagent when it is in the instrument. Bubble the gas through a gas dispersion tube at 3 L/minute for a minimum of 10 minutes.
- 3 To 100 mg of *o*-Phthalaldehyde (Cat. No. O120) in a clean, dry container add 10 mL of HPLC grade Methanol. Mix until homogeneous.
- 4 Remove the gas dispersion tube from the solution. Add the mixture to the deoxygenated Diluent in the reservoir. Wash any residual mixture into the reservoir with an additional 1–2 mL of Methanol.
- 5 Dissolve 2 g of Thiofluor in the reserved 5 mL of diluent and add into the degassed diluent in the reservoir.

**NOTE**

Once the reagent is mixed, securely capped and under inert gas pressure, do not bubble gas through the reservoir.

***OPA Reagent for Glyphosate Herbicide Analysis***

- Pour 945 mL of *o*-Phthalaldehyde (Cat. No. GA104) into the reservoir. Continue with steps 2-5 above.

***OPA Reagent for Amino Acid Analysis***

- Pour 945 mL of *o*-Phthalaldehyde Diluent (Cat. No. D104) into the reservoir. Continue with steps 2-5 above except use 300 mg OPA.
- Add 3 mL of 30 % aqueous Brij 35®†. Stir slowly or swirl gently to avoid foaming. When homogeneous, the reagent is ready for use. Brij 35 is necessary to prevent fluorescence quenching of the Lysine and Histidine derivatives.

**REAGENT RESERVOIR CONNECTIONS**

If the reagent is to be used in a Pickering Laboratories post-column instrument, the proper reservoir assembly, pump, and post-column connections will be in place.

The post-column system must be equipped with a means of maintaining a reagent reservoir pressure of 2 to 4 psi with an inert gas, typically N<sub>2</sub>. The tubing employed to connect the reservoir to the gas regulator, and to connect the reservoir to the pump must be air impermeable. For this reason, Air Barrier tubing is recommended over fluorocarbon (e.g., TFE, FEP) and polyolefin (-ethylene, -propylene) tubing. The tubing between the reagent pump and the mixing tee should be 1/16 inch OD x 0.030 inch ID Type 316 stainless steel or 1/16 inch OD x 0.020 inch ID PEEK.

**NOTE**

DO NOT USE fluorocarbon tubing with an air-sensitive reagent!

† A 30 % Brij 35 solution is available from Fisher Scientific Co., Cat No. BP 345-500.

Chromatographic Grade is a trademark of Pickering Laboratories, Inc.

Thiofluor is a trademark of Pickering Laboratories, Inc.

