INSTRUCTIONS

Fluoraldehyde (OPA) Reagent Solution

26025 0662.2

Number	Description
26025	**Fluoraldehyde (OPA) Reagent Solution,** 945mL, contains 0.8mg/mL of *o*-phthalaldehyde (OPA), pH 10
	CAS Number: 643-79-8
	Storage: Upon receipt store at 4°C away from oxidizing agents and direct sunlight. The reagent is best stored under a nitrogen head space. Adding small aliquots of 2-mercaptoethanol to the bottle after several weeks of use will extend the reagent's shelf-life. Product is shipped at ambient temperature.

Introduction

Thermo Scientific OPA (*o*-phthalaldehyde) is a primary amine-reactive fluorescent detection reagent that can be used as a protein/peptide assay reagent or as a post-column detection reagent for amino acid analysis (HPLC). Reaction of OPA with proteins and peptides yields linear results over a wide range of concentrations. The Fluoraldehyde Reagent Solution is supplied ready to use and enables fast quantitation of proteins or peptides in solution.

Important Product Information

- For best results, use a known quantity of purified sample protein/peptide as a standard. Alternatively, use a purified protein/peptide standard that has a similar response as the unknown sample or a purified standard protein such as bovine serum albumin (BSA).
- Primary amine-containing buffers such as Tris or glycine will interfere with OPA. Acetylated and other blocked peptides without primary amines on the amino acid side chains will not give a response with OPA. High molarity, low pH buffers may cause decreased fluorescence.
- Reducing agents and metal chelators do not interfere with this reagent, provided they are included in the blanks and standards. Also, most detergents do not interfere, and most common sample buffers and constituents are compatible.
- Fluorescence response with amino acids increases as the pH of the OPA solution is increased, except with histidine, which decreases fluorescence. Any pH value from 9.0 to 11.5 of the reagent will yield effective fluorescence.

Protocol for Protein/Peptide Assay

The standard protocol uses a ratio of 1:10 (sample:reagent). The sensitivity of the assay may be increased by using higher sample volume to reagent volume ratios such as 1:5, 1:1 or 10:1. If using a 96-well microplate, add 200µL of OPA reagent to 20µL samples within the wells. Use only opaque microplates that are designed for use with fluorescence assays.

1. Equilibrate the Fluoraldehyde Reagent Solution and samples to room temperature before use. The working range of protein/peptide concentration for the standard protocol is 10-500µg/mL.
2. Prepare a set of protein/peptide standards of known concentration by dissolving the standard protein/peptide in the same diluent as the unknown sample.
3. Add 200µL of sample, blank and standard to separate test tubes. Use the sample diluent as a blank.
4. Add 2mL of Fluoraldehyde Reagent Solution to each test tube and mix well.
5. Measure the fluorescence at excitation 330-390nm and emission at 436-475nm. Measure the fluorescence within 1-5 minutes. For best results, measure all samples at the same time interval after mixing.
6. Subtract the blank's fluorescence from the fluorescence emission values of the sample and standards to determine net fluorescence. Plot the net relative fluorescence of the standards versus concentration. Using the standard curve, determine the concentration of the unknown.

Amino Acid Assay (HPLC) Information

- Fluoraldehyde (OPA) Reagent Solution flow rate should equal that of column effluent. A 15-second delay coil between reagent addition point and fluorometer increases fluorescence. A 60-second delay coil further increases fluorescence for all amino acids except glycine, which is decreased by 20%. The delay coil can be maintained at room temperature.

Related Thermo Scientific Products

23225	**Pierce® BCA Protein Assay Kit**
23209	**Albumin Standard Ampules, 2mg/mL**, 10 × 1mL ampules, containing bovine serum albumin (BSA)
23208	**Pre-Diluted Protein Assay Standards: Bovine Serum Albumin (BSA) Set**, 7 × 3.5 mL
23212	**Bovine Gamma Globulin Standard, 2 mg/ml**, 10 × 1mL ampules
23213	**Pre-Diluted Protein Assay Standards**, (BGG) Set, 7 × 3.5mL aliquots
23235	**Pierce Micro BCA Protein Assay Kit**
23236	**Coomassie Plus (Bradford) Assay Kit**
23250	**Pierce BCA Protein Assay Kit–Reducing Agent Compatible**
20088	**Amino Acid Standard H,** 10 × 1mL
15042	**Pierce White Opaque 96-Well Plates,** 25/pkg
15075	**Reagent Reservoirs,** 200/pkg
15036	**Sealing Tape for 96-Well Plates,** 100/pkg

General References

Ogden, G. and Foldi, P. (1987). Amino acid analysis: An overview of current methods. *LC-GC* **5(1):**28-38.

Roth, M. (1971). Fluorescence reaction for amino acids. *Anal Chem* **43:**880-2.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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