

assays: protein

o-Phthalaldehyde (OPA) Fluorescent Protein Assay

OPA will react only with primary amines. When reacted with primary amines in the presence of mercaptoethanol, OPA yields an intense blue colored fluorescent product that has a maximum wavelength of excitation of 340 nm and emission at 455 nm.^{15,16} Wavelengths from 330-375 nm have been used for excitation and 436-490 nm for measuring emission. Protein concentrations as low as 50 ng/ml can be measured with an OPA assay. The inherent sensitivity and speed of OPA, along with its broad linear range, makes it a useful protein and peptide assay reagent.

OPA is ideal for assaying peptides that do not contain tyrosine residues, or for other applications in which absorbance at 280 nm cannot be used. Proteins and peptides tested yield linear results over a wide range of concentrations using both standard and microassay protocols.

There is considerable protein-to-protein and peptide-to-peptide variation with the OPA assay; therefore, it is best to use a purified sample of the particular protein or peptide as the standard. When this is not possible, the next best option is to use a protein or peptide that gives a response similar to the sample. Alternatively, a commonly accepted standard protein such as bovine serum albumin can be used.

Reducing agents and metal chelators do not interfere with an OPA-based assay, provided they are included in the blanks and standards. In addition, most detergents do not interfere. Any common sample buffers and constituents are also compatible, but primary amines such as Tris or glycine

buffers will interfere with OPA and must be avoided. Acetylated and other primary amine-blocked peptides will not give a response with OPA.

The Pierce Fluoraldehyde Protein/Peptide Assay is an *o*-phthalaldehyde-based reagent developed to detect minute amounts of protein and peptides. Fluoraldehyde reactions are complete in less than one minute with sensitivity down to 50 ng/ml. While some solutions interfere with protein/peptide measurement at 280 nm, the Pierce Fluoraldehyde Assay is compatible with many substances that interfere with other protein assays, such as detergents and reducing agents. Amine-containing buffers must be avoided, however, when performing assays using this chemistry.

In the standard assay mode, the Fluoraldehyde ready-to-use formulation can measure protein concentration in the range of 10 µg/ml-500 µg/ml, while the micro-assay working range is 50 ng/ml-25 µg/ml.

The Pierce Fluoraldehyde Protein/Peptide Assay Reagent requires only 200 µl of sample for use in a microplate assay, saving valuable sample and time. Fluoraldehyde assays require an excitation wavelength of 360 nm and emission wavelength of 455 nm.

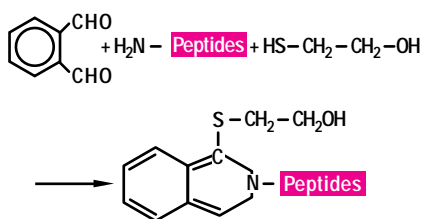


Figure 6: The reaction of *o*-Phthalaldehyde with a primary amine on a peptide in the presence of 2-Mercaptoethanol to form a fluorescent-labeled peptide.

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, 880-882.