

FT-02727B

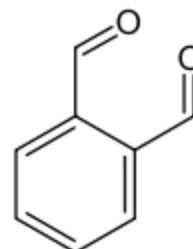
OPA, amine detection reagent

Sensitive fluorescent detection reagent for amines (i.e. aa, proteins and peptides)

Works also by absorbance; Can also be used for thiols detection.

Product Description

Part number: 02727B 5g 02727C 25g
 Chemical Name: **o-Phthalaldehyde (OPA)**
 Properties: CAS 643-79-8 ; MW=134.12
 λ excitation = 340nm, λ emission = 455nm
 Storage : +4°C for long term (possible at room Temperature),
 protected from light and moisture (H)



OPA reagent provides a very high sensitivity detection reagent of amines, notably contained in proteins, peptides, and aminoacids. It can also be used to quantitate thiols.

Uptima offers a highly purified OPA for best results in HPLC, capillary electrophoresis, and spectrophotometric assays of protein/peptide and amino-acids.

Scientific & technical Information

- OPA is well soluble, and stable in water solution at pH<11.5. It is however sensitive to UV illumination and air oxidation.
- In adequate conditions, OPA reacts in presence of thiols specifically with primary amines above their isoelectric point P_i . The reaction during 1 minute (less for glycine). The reaction starts rapidly and can be monitored by absorbance, and by fluorescence. The formed derivates are however not stable. Absorbance at 340nm increase within 15seconds up to 1-3 minutes, then decreases more or less slowly depending on compounds (glycine, peptides,...). I.e. AcetylCysteine maximum absorbance is maintained between 1' and 1'30. One mole of ϵNH_2 gives on OD340nm of approximately 10 units. Acetone and Dioxane don't affect the assay.

A more sensitive detection is achieved by fluorescence, with excitation at 330-390nm (max.340nm), and measurement at 436-475nm (max 455nm). There are noticeable variations of the fluorescent signal between amino-acids, and fluorescence might increase with pH values (not for histidine). Thus, it is recommended for accurate and sensitive results to use a purified standard homologous of the molecule of interest, to eventually optimize reaction duration and pH (between 9 and 11.5).

- OPA reacts also with thiols in presence of an amine such as n-propylamine or 2-aminoethanol.

[Protein and peptide assay](#) | [Amino-Acids detection / amines](#) | [Amino-Acids detection / thiols](#)

Directions for use

Here are 2 standard protocols. Conditions should be optimized for specific applications, notably the duration of incubation depending on amino acid or aa-content of peptides/proteins.

Protocol: Protein and peptide assay ^[1]

This protocol is designed to measure amine content in peptides and proteins to control the degree of labeling or conjugation (differential amine content before and after labeling). α -acetyl-lysine can be used as standard, containing 1 amine per molecule. A ratio of labeling can then be determined.

➤ Preparation:

- Prepare a 50mM carbonate pH10.5 buffer
- Prepare the working reagent : 5mg of OPA + 100 μ l of pure Ethanol + 5 μ l of b-2-mercatoethanol + 10ml of 50mM carbonate buffer pH10.5

Note: This OPA reagent should be protected from direct light, and used within 2 hours. It can eventually be stored under nitrogen in ambered glass vials for 1-2 weeks at 4°C.

- Prepare the standards:

Prepare a 10mM acetylLysine (09111A) standard solution in water (18.8mg α acLys + 10ml dH₂O).

Prepare serial dilutions of 800nM to 12 μ M of α acLys standard solution in carbonate buffer.

Rem : for accurate quantitation, a standard curve may be prepared with a purified peptide or protein

- Prepare samples dilutions in carbonate buffer.

➤ Assay:

- Pipette 100 μ l of sample in clean disposable tubes.

Rem : plastic tubes of bad quality may produce background signal.

Check that OD of a blank is quite stable within 0-5min

- Add 1ml of OPA reagent (1-), incubate at room temperature for 2min:

- Place the solution in spectrophotometer, and read absorbance at 340nm at exactly time, i.e. 2min

Rem : The duration of incubation could be set up with the molecule of interest.

Rem : readings should be performed after the same incubation duration for greater accuracy. ODs are sufficiently stable

between 1min and 2minutes for α acLys and 2-4min for proteins. The incubation period may so be optimized, down 1min30 and up 10min, depending of proteins and standard (same known protein is better).

Rem : the solution should be put in the spectrophotometer just before reading, because continuous exposure to UV affects the signal. One sample can be prepared during the incubation of the previous sample.

- Plot a standard curve of amine detection, with the molar concentration of standard in x-axis and ODs on y-axis, then calculate for each sample the corresponding amine concentration.

Calculate the sample concentration taking in account the dilution factor.

➤ Interpretation:

A sensitivity of 10 μ M can be obtained by absorbance measurement.

For higher sensitivity, the concentration of OPA can be increased.

This protocol could be adapted to microplates. Use transparent plates for working in absorbance. The OPA reagent distribution in wells and OD reading should be well timed, e.g. use 100 μ l of sample, read at 340nm (blank), add 100 μ l of OPA reagent, and read again at 340nm.

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This protocol can be adapted to fluorescent measurement with a fluorimeter ⁰. A wide dynamic range of detection can be achieved (15-1000µg/ml of protein), or very sensitive but shorter range (1-50µg/ml) by increasing up 20 fold the OPA/protein ratio.

References for Peptide and protein spectrometric or fluorometric assay

Bertrand-Harb C., Nicolas M.G., Dalgalarraondo M., Chobert J.M., Determination of alkylation degree by three colorimetric methods and amino-acid analysis. A comparative study, *Science des aliments* 1993, 13, 577-584

Jones, B.N. and Gilligan, J.P. (1983). *American Biotechnology Laboratory*, December Issue, 46-51.

Bowden K. et al.; *J. Chem. Soc., Perkin Trans. II*, 12, 2089-2092 (1990). The intramolecular Cannizzaro reaction of o-phthalaldehyde

Vanden Eynde J.-J. et al. ; *Bull. Soc. Chim. Belg.* 101, 6, 529-12(1992): Novel syntheses of heterocycles with N-(1-haloalkyl)azinium halides

Protocol: Amino-Acids / amines detection ^[2]

- prepare a fresh solution of 70mg OPA + 1ml Methanol + 95ml of buffer pH10.5 (25g/L of Boric acid (UP07044), 0.3% Brij™35 (UP09187), 0.2% 2-mercaptoethanol). Purge with N₂ and store in the dark (stable for 1-2 weeks).
- Pre-column derivatization is recommended for optimum sensitivity. Inject the OPA reagent with the sample before the chromatographic separation (2-fold volume excess). Agitate for 1 minute, and inject onto column. For post column derivatization, use flow rate of OPA reagent equal to eluant).

A sensitivity of low picomole range can be obtained. See references below.

Guide line for use: Thiols detection ^[3]

Thiol reaction with OPA require the presence of an amine, such as n-propylamine or 2-aminoethanol.

References for using OPA in thiol determination

- Determination of hydrophilic thiols in sediment porewater using ion-pair liquid chromatography coupled to electrochemical detection; Damian Shea, William A. MacCrehan; *Anal. Chem.*, 1988, 60 (14), pp 1449-1454; [Abstract](#)

"phthalaldehyde (OPA) in the presence of excess amine (2- aminoethanol) has been used to determine thiols in a pore- water sample of reducing sediment"

- Trace determination of biological thiols by liquid chromatography and precolumn fluorometric labeling with o-phthalaldehyde; Kenneth Mopper, Daniel Delmas; *Anal. Chem.*, 1984, 56 (13), pp 2557-2560; [Abstract](#)

"Trace Determination of Biological Thiols by Liquid ... prior to derivatization with OPA and 2-aminoethanol."

- Chromatographic Determination of Thiols After Pre-column Derivatization with o-Phthalaldehyde and Isoleucine ; V. Concha-Herrera; J. R. Torres-Lapasi; M. C. Garca-Alvarez-Coque; [Abstract](#)

Journal of Liquid Chromatography & Related Technologies, Volume 27, Issue 10 January 2005 , pp 1593 - 1609

"reaction of primary amines with excess o-phthalaldehyde (OPA) and thiol yields unique isoindole derivatives that are readily separated by reversed-phase liquid chromatography"

- Determination of glutathione and glutathione disulfide in biological samples: An in-depth review ;

Péter Monostoria, Gyula Wittmann, Eszter Karga and Sándor Túri; [Abstract](#)

Journal of Chromatography B Volume 877, Issue 28, 15 October 2009, Pages 3331-3346

- Field method for determination of traces of thiols in natural waters ; Appathurai Vairavamurthy1, and KMopper.

Analytica Chimica Acta Volume 236, 1990, Pages 363-370; [Abstract](#)

"the thiols can be regenerated from DTNP derivatives using tributylphosphine (TBP) and derivatized with o-phthalaldehyde"

- Fluorometric Determination of Cyanide with o-Phthalaldehyde and Taurine ; [Article](#)

Analytic

P.3

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al Sciences Vol.2 , No.5(1986)pp.491-492; Akira SANO, Masaaki TAKEZAWA and Shoji TAKITANI

"reaction with o-phthalaldehyde and 2-aminoethanol, to isoin- Trace determination of biological thiols by liquid chromatography and precolumn fluorometric "

Guide lines for use: Derivatisation for Chromatography [4]

References for Chromatography applications

Gardner W., Miller W., 1980, Reverse-phase liquid chromatography analysis of amino acids after reaction with o-phthalaldehyde. Analytical Biochemistry, 1980, 101, 61-65

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

Roth M., Fluorescence reaction for amino acids, Anal.Chem., 1971, 43, 880-882

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Godel, H., et al. (1984). J. Chrom. 297, 49-61.

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Krishnamurti, C.R., et al. (1984). J. Chrom. 315, 321-331.

Jones, B.N., et al. (1983). J. Chrom. 266, 471-482.

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Benson, J.R. and Woo, D.J. (1984). J. Chromatogr. Sci. 22, 386-399.

Related products – fluorescent derivatization reagents

1,8-Diazfluoren-9-one #HH6521 : Fluorescent derivatization of amino-acids ($\lambda_{exc}/\lambda_{em}$ = ~470/~570 nm)

Ninhydrin # 024400: Fluorescent derivatization of amino-acids

NBD-F # FP-U0573A: labeling of amines (aliphatic, aa, peptides, proteins)

NHS ester of all conventional fluorophores (coumarin, fluorescein, rhodamine... ; [FluoProbes](#): labeling agents for amines)

ABD-F #FP-57564A: labeling of thiols (30times faster than SBD-F; ideal for TLC, HPLC)

SFB-F #FP-AM859A and SFB-Cl #FP-AM858A: labeling of thiols, widely used for chromatography

DTNB #015661 (amine and sulfhydryl assay reagent)

DMEQ-COCl #FP-69129A (labeling of primary and secondary alcohols)

APTS #FP-33972A (labeling of aldehyde and ketones (of glycoproteins or sugars) for HR CE)

ANTS #FP-46574A (labeling of aldehyde and ketones (of glycoproteins or sugars) for sequencing and CE)

4NBA #BL9650, 2HP #O19022, 2SBA #A42050 (reagents to assay and block aldehydes and hydrazine)

DNP #015160 (Reagent for the determination of aldehydes and ketones)

3-Mercaptopropionic acid (3MPA) #021590, nucleophile for most stable GABA

Other information

See [BioSciences Innovations catalogue](#) and [e-search tool](#).

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