OPA, amine detection reagent

Sensitive fluorescent detection reagent for amines (i.e. aa, proteins and peptides) (10µM, to 10pmol/GC)
Works also by absorbance; Can also be used for thiol detection.

Product Description

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

Introduction

OPA is a very high sensitivity detection reagent of amines, notably contained in proteins, peptides, and aminoacids.
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 Pi. The reaction during 1 minute (less for glycine).
• The reaction starts within 15 seconds 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. I.e. AcetylCysteine maximum absorbance is maintained between 1’ and 1’30. One mole of εNH2 gives on OD340nm of approximately 10 units. Acetone and Dioxane don’t affect the assay.
A better detection can be achieved by fluorescence, with excitation at 330-390nm (max.340nm), and measurement at 436-475nm (max 455nm). The fluorescence increases with pH (excepted for Histidine).
• There are noticeable variations of the fluorescent signal between amino-acids, and fluorescence might increase with pH values. 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.

Directions for use

Protein and peptide assay | Amino-Acids detection / amines | Amino-Acids detection / thiols

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 1 : Protein and peptide assay
This protocol is designed to measure amine content in peptides and proteins before and after derivatization by biotinylation or cross-linking agents. One could use the standard α-acetylt-lysine, containing 1 amine per molecule. A ratio of derivatization can then be determined.

1- Prepare a 50mM carbonate pH10.5 buffer

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

3- Prepare a 10mM acetylLysine (UP09111) standard solution in water (18.8mg αacLys + 1ml dH2O)

4- Prepare serial dilutions of 800 to 12µM of αacLys standard solution in carbonate buffer.
   Rem : for accurate quantitation, a standard curve may be prepared with a purified peptide or protein

5- Prepare samples dilutions in carbonate buffer

6- Pipette 100µl 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

7- Add 1ml of OPA reagent (1-), incubate at room temperature exactly during 1min and 30 seconds
   rem: a 2min incubation may be preferred if a protein standard is used)

8- Place the solution in spectrophotometer, and read absorbance at 340nm
   Rem: the solution should be put in the spectrophotometer just before reading, because continuous exposure to UV affect the signal.
   rem: One sample can be prepare during the incubation of the previous sample. Readings should be performed after the same duration for greater accuracy, 1min and 30 seconds exactly, although ODs are normally sufficiently stable between 1min and 2minutes for αacLys.
   Rem: The duration of incubation could be sat up with the molecule of interest.

9- 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.

A sensitivity of 10µM can be obtained with absorbance measurement. For higher sensitivity, the concentration of OPA can be increased.

This protocol can be adapted to fluorescent measurement with a fluorimeter.
This protocol could be adapted to microplates (transparent plates for working in absorbance, or opaque black plates for working in fluorescence) provided the OPAreagent distribution in wells and OD reading are performed rapidly enough and with similar speed.: use 100µl of sample, read at 340nm (blank), and 100µl of OPA reagent), and read again at 340nm.
Protocol 2 : Amino-Acids detection / amines

1- 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).

2- 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.

Guide line for use 3: Amino-Acids detection / thiols

Thiol reactions require the presence of an amine, such as n-propylamine or 2-aminoethanol

References for using OPA in thiol determination in presence of e.g. 2-aminoethanol:

Determination of hydrophilic thiols in sediment porewater using ion-pair liquid chromatography coupled to electrochemical detection; Damian Shea, William A. MacCrehan; Abstract Anal. Chem., 1988, 60 (14), pp 1449–1454
"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; Abstract Anal. Chem., 1984, 56 (13), pp 2557–2560
"Trace Determination of Biological Thiols by Liquid ... prlor to derivatization with OPA and 2-aminoethanol.

"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 (OPA). "

Fluorometric Determination of Cyanide with o-Phthalaldehyde and Taurine ; Article Analytical Sciences Vol.2 , No.5(1986)pp.491-492; Akira SANO, Masaaki TAKEZAWA and Shoji TAKITANI "reaction with o-phthalaldehyde and 2-aminoethanol, to isoindol- .... Trace determination of biolog- ical thiols by liquid chromatography and precolumn fluorometric "

Other information

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References

Peptide and protein spectrometric or fluorometric assay

Chromatography applications


Related products – fluorescent derivatization reagents
See BioSciences Innovations catalogue and e-search tool.

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

1,8-Diazafluoren-9-one #HH6521 : Fluorescent derivatization of amino-acids (λexc./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... based)
NHS ester of all our great FluorProbes labels (labeling agents for amines)

ABD-F #FP-57564A: labeling of thiols (30times faster than SBD-F; ideal for TLC, HPLC)
SFBA-F #FP-AM859A and SFBA-Cl #FP-AM858A: labeling of thiols, widely used for chromatography
DTNB #015661 (amine and sulfhydryl assay reagent)

DMEQ-COCI #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)

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