

OPA, amine detection reagent

Product Description

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

Catalog number: UP02727A OPA, 1g
Chemical Name: o-Phthalaldehyde
CAS 643-79-8, C₈H₆O₂; M.W.=134.12
 λ excitation = 340nm, λ emission = 455nm
Storage : Room Temperature, protected from light and moisture

General Information

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 and 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 starts within 15 seconds and increases during 1 minute (less for glycine).
- The reaction can be monitored by absorbance at 340nm. The formed derivatives are however not stable, and absorbance decreases more or less slowly. AcetylCysteine maximum absorbance is maintained between 1' and 1'30. One mole of ϵ NH₂ 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 is noticeable variations of the fluorescent signal between amino-acids, thus it is recommended for accurate results to use a purified standard homologous of the molecule of interest. The highest sensitivity of detection occurs after 1-4minutes of incubation.
- OPA reacts also with thiols in presence of an amine such as n-propylamine or 2-aminoethanol.

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..

Protocole 1 : Protein and peptide assay

This protocole is designed to control amine content in peptides and proteins before and after derivatization by biotinylation or cross-linking agents. One could use the standard α -acetyl-lysine, containing 1 amine par molecule. A ratio of derivatization can then be determined.

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FT-UP02727

- 1- Prepare the following reagent :
5mg of OPA + 100µl of pure Ethanol + 5µl of b-2-mercaptoethanol + 10ml of 50mM carbonate buffer pH10.5
This OPA reagent should be protected from direct light, and used within 2 hours
- 2- Prepare a 10mM acetylLysine (UP09111) standard solution in water (18.8mg αacLys + 1ml dH2O)
- 3- 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
- 4- Prepare samples dilutions in carbonate buffer
- 5- 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
- 6- Add 1ml of OPA reagent (1-), incubate at room temperature exactly during 1min and 30 secondes
rem: a 2min incubation may be preferred if a protein standard is used)
- 7- 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.
- 8- 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 protocole can be adapted to fluoresent measurement with a fluorimeter.

This protocole 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.

Protocole 2 : Amino-Acids detection

- 1- prepare a fresh solution of 70mg OPA + 1ml Methanol + 95ml of buffer pH10.5 (2.5g/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. Inject the OPA reagent with the sample before the chromatographic separation (2-fold volume excess), or after separation prior to UV detection. Agitate for 1 minute, and inject onot column. For post column derivatization, OPA gives the highest sensitivity of any derivatization reagents.

A sensitivity of low picomole range can be obtained

Other Information

For any information, please contact Uptima - Interchim, 213 av.J.F.kennedy, 03103 Montlucon, fax :+33 4 70 03 82 60, hotline Interbiotech : +33 4 70 03 76 06 :

Literature Peptide and protein spectrometric or fluorometric assay

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Chromatography applications

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