

PCAS-H4 -D4**Product Information**

PCAS-H4 -D4

PyridineCarboxylicAcidSuccinimide

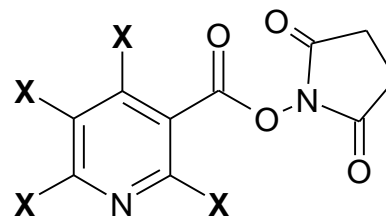
8 x 1 mg of PCAS-H4 Δ0

8 x 1 mg of PCAS-D4 Δ4

Cat. Number: 011S

Formula: C₁₀H₈N₂O₄ / C₁₀D₄H₄N₂O₄

Molecular Weight: 220 / 224

**X=H - PCAS-H4****X=D - PCAS-D4**

Features:

Isotopically-coded.

Amino group reactive.

Inter-peptide crosslinks identification.

Differential protein surface modification.

Relative protein quantitation.

PCAS-H4 -D4 is an isotopically-coded, amino group reactive modification reagent Pyridine-3-Carboxylic Acid Succinimide. Light (H4) and heavy (D4) forms of the reagent differ by 4 deuterium atoms in heavy form instead of 4 hydrogen atoms of light form, and otherwise are chemically identical. Isotopic coding enables univocal detection of the PCAS-modified products in mass spectra. Reaction products of PCAS-H4/D4 will manifest in mass spectra as doublets of peaks of equal intensity corresponding to light (H4) and heavy (D4) forms of the reagent separated by 4.02508 Da divided by charge state (4.02 for +1, 2.01 for +2, 1.34 for +3 etc.).

PCAS-H4 -D4 can be used as N-terminal modification reagent for the specific detection of the inter-peptide crosslinks (Petrotchenko et al., 2010). Crosslinked proteins digest is split in half and each half is modified with PCAS-H4 or PCAS-D4, respectively. Reaction mixtures are quenched with ammonium bicarbonate, combined and analyzed by mass spectrometry. Inter-peptide crosslinks will manifest in mass spectra as doublets of signals separated by 8.05 Da, as they contain two N-termini, and non-crosslinked peptides will manifest as doublets of signals separated by 4.02 Da, as they contain one N-terminus. Alternatively, crosslinked protein digest can be modified with an equimolar mixture of PCAS-H4/D4. In this case doubly-modified inter-peptide crosslinks will manifest in spectra as triplets of signals separated by 4.02 Da with 1:2:1 intensity ratio. It should be noted, that lysine-containing non-crosslinked peptides will be also doubly modified and, therefore, exemplify the same mass spectrometric signatures as inter-peptide crosslinks.

PCAS-H4 -D4 can be used as reagent for differential protein surface modification studies. Protein in two different states can be modified with PCAS-H4 or PCAS-D4, respectively. Reactions are quenched with ammonium bicarbonate, combined, digested and analyzed by mass spectrometry. Peptides, carrying modified sites, will manifest in spectra as doublets of signals separated by 4.02 Da with intensity ratios for light (H4) and heavy (D4) forms proportional to the reactivity of the modified amino acid residues in two different states of the protein. Thus, equally modified peptides will result in doublets of signals of approximately the same intensity, while differentially modified peptides will result in doublets of signals with different intensities for light (H4) and heavy (D4) forms. In addition to N-terminal and lysine amino groups, the PCAS reagent also modifies in lesser extent Tyr, Ser, Thr and His residues. To additionally analyze modification of these residues, enzymatic digestion of the modified proteins should be performed under low pH conditions, for example with pepsin (Serpa et al., 2013).

PCAS-H4 -D4 also can be used as reagent for differential protein quantitation studies, similar to AB Sciex mTRAQ reagent. Two protein digest samples are modified with PCAS-H4 or PCAS-D4, respectively, quenched, combined and analyzed by mass spectrometry. Peptides, derived from the proteins, which present in samples in different amounts, will manifest in spectra as doublets of signals with different intensities for light (H4) and heavy (D4) forms (Münchbach et al., 2003). It should be noted, that light (H4) and heavy (D4) forms of the modified peptides will elute little differently in reversed phase HPLC, with heavy (D4) form eluting little earlier.

N-HydroxySuccinimide (NHS) esters react mainly with primary amino groups (-NH₂) in pH 7-9 buffers to form stable amide bonds. Therefore, amine-containing buffers (Tris, Glycine, ammonium salts, etc.) should be avoided for modification reaction. PCAS is water-insoluble and stock solutions should be prepared in an organic solvent such as DMSO or DMF and then added to the aqueous reaction mixture. To make 100 mM stock solution of the PCAS-H4 -D4, add 45 μl DMSO to the pre-weigh tube containing 1 mg of the reagent.

To calculate masses of modified peptides use following formulas:

$$[M_1\text{-PCA}_{\text{H4}}+\text{H}]^+ = [M_1+\text{H}]^+ + 105.02146$$

$$[M_1\text{-PCA}_{\text{D4}}+\text{H}]^+ = [M_1+\text{H}]^+ + 109.04812$$

$$[M_1\text{-PCA}_{\text{H4}}\text{-PCA}_{\text{H4}}+\text{H}]^+ = [M_1+\text{H}]^+ + 210.04293$$

$$[M_1\text{-PCA}_{\text{D4}}\text{-PCA}_{\text{D4}}+\text{H}]^+ = [M_1+\text{H}]^+ + 218.09624$$

, where M_1 - mass of peptide; $M_1\text{-PCA}$, $M_1\text{-PCA-PCA}$ – mass of singly and doubly modified peptide, respectively.

MS-Bridge (<http://prospector.ucsf.edu>) modification elemental composition: C6 H3 N O.

Typical MALDI mass spectrum of the test reaction of the peptide (SSDTYRYISSKSSS) with PCAS-H4/D4 is shown in Figure 1. Masses of the reaction products for the light (H4) form of the reagent are: 1567 – free peptide; 1672 – singly modified peptide; 1777 – doubly modified peptide.

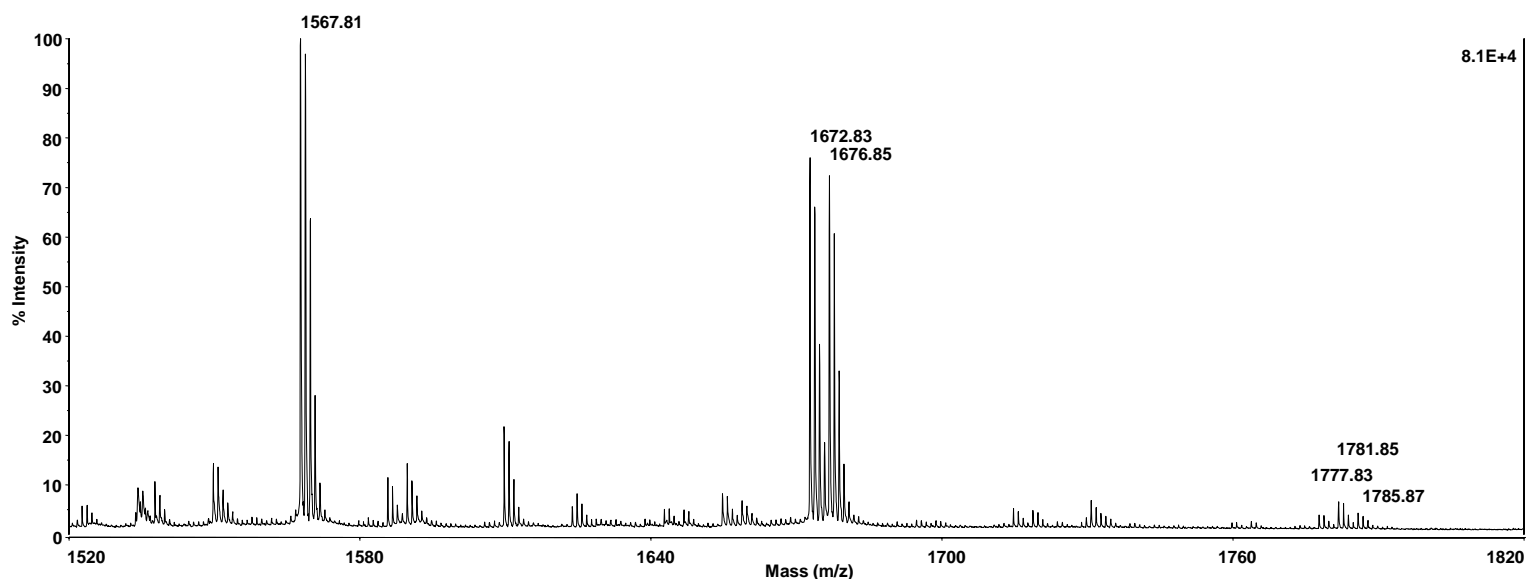


Figure 1. Mass spectrum of reaction products of the peptide modified with PCAS-H4/D4.

Material Safety Data information: substance is not fully tested yet.

References:

Petrotchenko EV, Serpa JJ, Borchers CH. Use of a combination of isotopically coded cross-linkers and isotopically coded N-terminal modification reagents for selective identification of inter-peptide crosslinks. *Anal Chem.* 2010; 82(3):817-23.

Serpa JJ, Patterson AP, Pan J, Han J, Wishart DS, Petrotchenko EV, Borchers CH. Using multiple structural proteomics approaches for the characterization of prion proteins. *J Proteomics.* 2013; 81:31-42.

Münchbach M, Quadroni M, Miotto G, James P. Quantitation and facilitated de novo sequencing of proteins by isotopic N-terminal labeling of peptides with a fragmentation-directing moiety. *Anal Chem.* 2000; 72(17):4047-57.