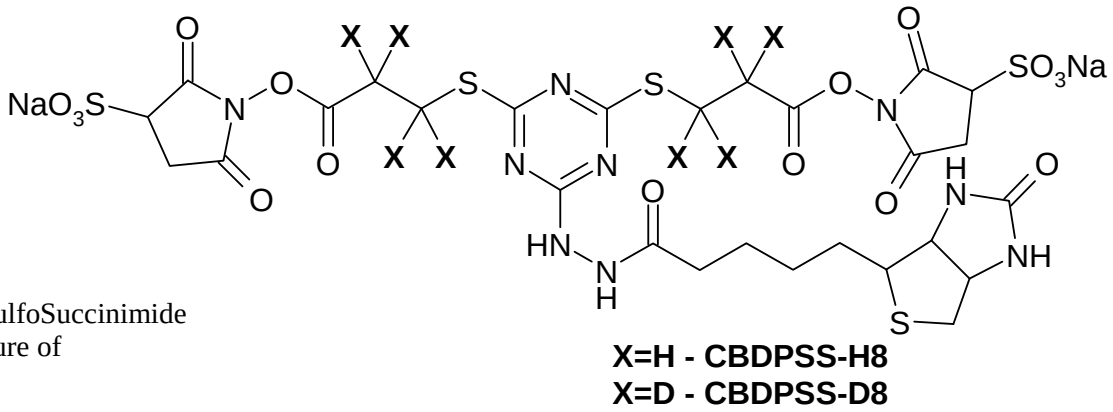
CBDPSS-H8/D8

Product Information



CBDPSS-H8/D8
CyanurBiotinDimercaptoPropionylSulfoSuccinimide
12 x 0.94 mg of 1:1 molar ratio mixture of
CBDPSS-H8 and CBDPSS-D8
Cat. Number: 014SS
Formula: $C_{27}H_{31}N_9Na_2O_{16}S_5$ / $C_{27}D_8H_{23}N_9Na_2O_{16}S_5$
Molecular Weight: 943 / 951

Features:
Isotopically-coded.
Biotinylated.
CID-cleavable.
Isotopic MS/MS signatures for dead-end and inter-peptide crosslinks.
Water-soluble.

CBDPSS-H8/D8 is a water-soluble, homobifunctional, isotopically-coded, affinity-tagged, CID-cleavable crosslinker CyanurBiotinDimercaptoPropionylSulfoSuccinimide. Light (H8) and heavy (D8) forms of the reagent differ by 8 deuterium atoms in heavy form instead of 8 hydrogen atoms of light form, and otherwise are chemically identical. Isotopic coding enables univocal detection of the crosslinked products in mass spectra. Reaction products of CBDPSS-H8/D8will manifest in mass spectra as doublets of peaks of equal intensity corresponding to light (H8) and heavy (D8) forms of the reagent separated by by 8.05016 Da divided by charge state (8.05 for +1, 4.03 for +2, 2.68 for +3 etc.).

CBDPSS-H8/D8 is biotinylated and therefore CBDPS crosslinks are affinity purifyable with immobilized avidin or streptavidin. Affinity enrichment of the crosslinks allows to eliminate interfering free peptides from the analysis and to enhance detection of the crosslinks.

CBDPSS-H8/D8 is CID cleavable. In combination with isotopic coding it creates specific signatures for the cleavage products of the CBDPS crosslinks in MS/MS spectra (Ref. 1).

N-HydroxySulfoSuccinimide (NHSS) esters react mainly with primary amino groups ($-NH_2$) in pH 7-9 buffers to form stable amide bonds. Therefore, amine-containing buffers (Tris, Glycine, ammonium salts, etc.) should be avoided for crosslinking reaction. CBDPSS is water-soluble and stock solutions can be prepared in water. To make 100 mM stock solution of the CBDPSS-H8/D8, add 10 μl water to the pre-weigh tube containing 0.94 mg (1 μmol) of the reagent.

To calculate masses of peptide crosslinks use following formulas:
$[M_{12}+H]^+ = [M_1+H]^+ + [M_2+H]^+ + 508.08899$
$[M_1OH+H]^+ = [M_1+H]^+ + 527.10738$
$[M_1i+H]^+ = [M_1+H]^+ + 509.09682$
$[M_1NH_2+H]^+ = [M_1+H]^+ + 526.12336$
, where M_1, M_2 - masses of free peptides; M_{12} – mass of inter-peptide crosslink; M_1OH – mass of dead-end crosslink; M_1i – mass of intra-peptide crosslink; M_1NH_2 – mass of dead-end amide (if reaction was quenched with ammonium salts).
MS-Bridge (http://prospector.ucsf.edu) bridge elemental composition: C19 H23 N7 O4 S3; modification elemental composition for –OH dead-ends: C19 H25 N7 O5 S3; modification elemental composition for $–NH_2$ dead-ends: C19 H25 N8 O4 S3.

Typical MALDI mass spectrum of the test reaction with FLAG (DYKDDDDK) peptide is shown in Figure 1. Masses of the reaction products for the light (H8) form of the reagent are: 1013 – free FLAG peptide; 1522 – intra-peptide crosslink; 1540 – dead-end crosslink; 2534 – inter-peptide crosslink.

CBDPSS-H8/D8 crosslinks can be cleaved by CID (Figure 2). Cleaved moieties of the crosslink still isotopically labeled with H4/D4 atoms and will manifest in MS/MS spectra as doublets of peaks of equal intensity separated by 4.03 Da divided by charge state. As the cleavage can occur equally at either of two C-S bonds of the crosslinker, it results in two sets of H4/D4 doublets separated by 401 Da for each individual peptide constituting inter-peptide crosslink (Figure 2), (Ref. 1).

Cleaved crosslinks masses can be calculated using following formulas:
$[M_{12}+H]^+$ = $[M_1cl+H]^+ + [M_2cl+H]^+ + Mcliploss$
$[M_1OH+H]^+$ = $[M_1cl+H]^+ + Mclohloss$
$[M_1i+H]^+$ = $[M_1icl+H]^+ + Mcliloss$
$[M_1cl+H]^+$ = $[M_1+H]^+ + Mclrest$
$[M_1icl+H]^+$ = $[M_1+H]^+ + Mclirest$
, where H – mass of proton; M_1, M_2- masses of free peptides; M_{12} – mass of inter-peptide crosslink; M_1OH – mass of dead-end cleaved intra-peptide crosslink; Mcliploss, Mclohloss, Mcliloss – mass additions for cleaved inter-peptide, dead-end and intra-peptide crosslinks, correspondently; Mclrest, Mclirest – mass of cleaved portion of the crosslinking reagent for cleaved inter-peptide or dead-end and intra-peptide crosslinks, correspondently (Table 1).

Table 1. Mass additions for CBDPS crosslinks CID cleavage products.

Reagent	Cleavage	clrest el. comp.	Mclrest	Mclirest	Mcliploss	Mclohloss	Mcliloss
CBDPS	CID	C16 H21 N7 O3 S3	455.08625	-	-1.00727	72.02058	-
		C3 H2 O1*	54.01002	-	-1.00727	473.09682	-

* - tentatively CID cleavage of proximal C-S bond produces ion of structure P_1-CO-CH_2-CH_2^+, where P_1 – peptide moiety.

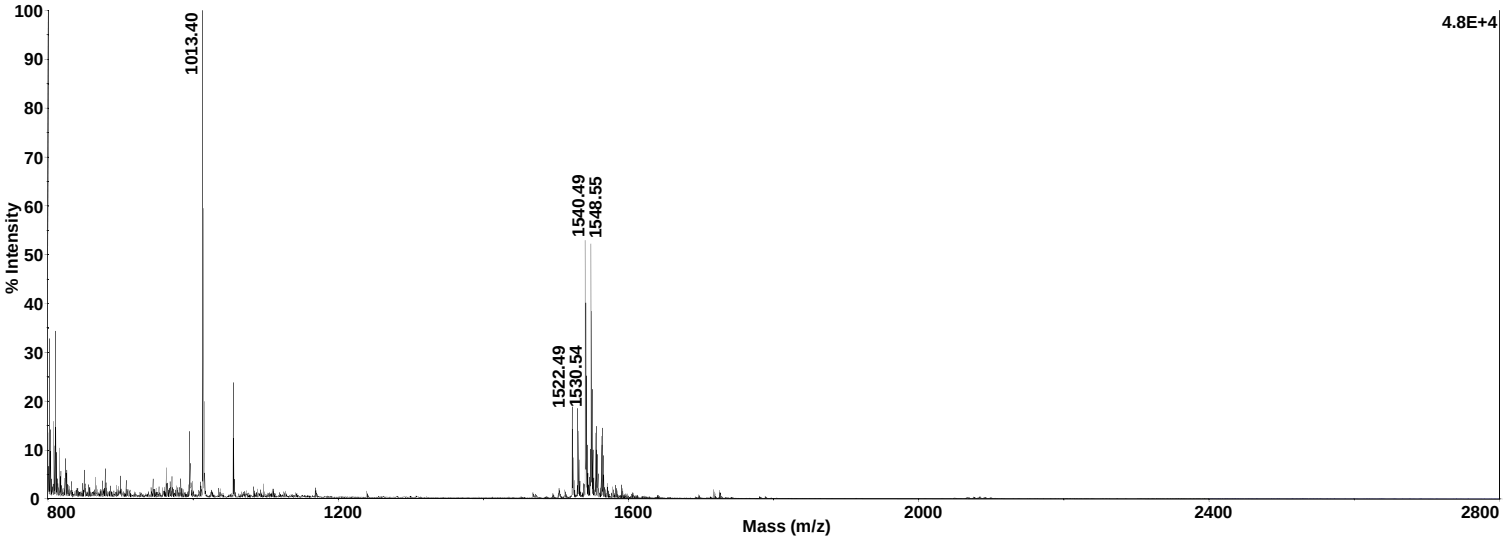


Figure 1. Mass spectrum of reaction products FLAG peptide modified with CBDPS-H8/D8.

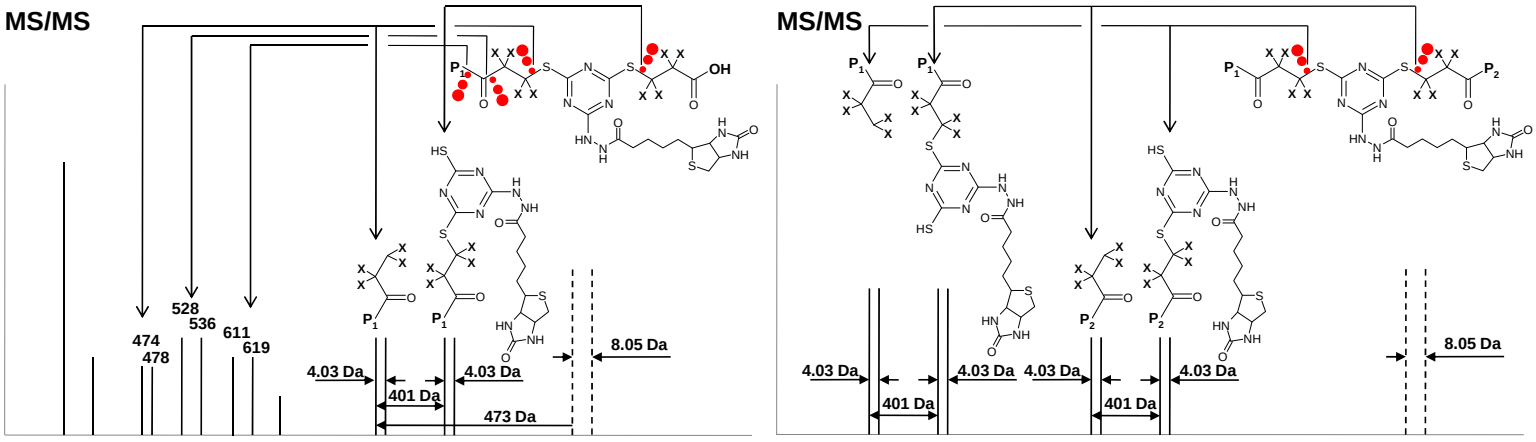


Figure 2. Scheme of CID cleavage of CBDPSS-H8/D8 dead-end (left panel) and inter-peptide (right panel) crosslinks.

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

References:
1. Petrotchenko EV, Serpa JJ, Borchers CH. An isotopically-coded CID-cleavable biotinylated crosslinker for structural proteomics. Mol Cell Proteomics. 2011 Feb;10(2):M110.001420.

