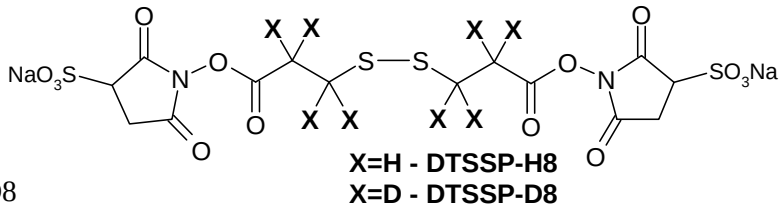
DTSSP-H8/D8

Product Information



DTSSP-H8/D8
Di-ThioSulfoSuccinimidylPropionate
12 x 1 mg of 1:1 molar ratio mixture of DTSSP-H8 and DTSSP-D8
Cat. Number: 002SS
Formula: $C_{14}H_{14}N_2Na_2O_{14}S_4$ / $C_{14}D_8H_6N_2Na_2O_{14}S_4$
Molecular Weight: MW 608 / 616

Features:
Isotopically-coded.
Water-soluble.
Chemically cleavable.
CID cleavable.

DTSSP-H8/D8 is a water-soluble, homobifunctional, isotopically-coded cleavable crosslinker Di-ThioSulfoSuccinimidyl-Propionate. 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 DTSSP-H8/D8 will manifest in mass spectra as doublets of peaks of equal intensity corresponding to light (H8) and heavy (D8) forms of the reagent separated by 8.05016 Da divided by charge state (8.05 for +1, 4.03 for +2, 2.68 for +3 etc.).

N-HydroxySulfoSuccinimide (NHS) 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. DTSSP is water-soluble and stock solutions can be prepared in water. To make 50 mM stock solution of the DTSSP-H8/D8, add 33 µl water to the pre-weigh tube containing 1 mg of the reagent.

To calculate masses of peptide crosslinks use following formulas:
$[M_{12}+H]^+ = [M_1+H]^+ + [M_2+H]^+ + 172.97310$
$[M_1OH+H]^+ = [M_1+H]^+ + 191.99149$
$[M_1i+H]^+ = [M_1+H]^+ + 173.98093$
$[M_1NH_2+H]^+ = [M_1+H]^+ + 191.00747$
, 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: C6 H6 O2 S2; modification elemental composition for –OH dead-ends C6 H8 O3 S2; modification elemental composition for $–NH_2$ dead-ends: C6 H9 N1 O2 S2.

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; 1187 – intra-peptide crosslink; 1205 – dead-end crosslink; 2199 – inter-peptide crosslink.

DTSSP-H8/D8 crosslinks can be cleaved by DTT (Figure 2) (Ref. 1) or CID (Figure 3). In both cases cleaved moieties of the crosslink still isotopically labeled with H4/D4 atoms and will manifest in spectra as doublets of peaks of equal intensity separated by 4.03 Da divided by charge state. As in case of CID, 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 66 Da for each individual peptide constituting inter-peptide crosslink (Figure 3) (Ref 2,3).

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 DTSP crosslinks cleavage products.

Reagent	Cleavage	clrest el. comp.	Mclrest	Mclirest	Mcliploss	Mclohloss	Mcliloss
DTSP	DTT	C3 H4 O1 S1	87.99829	175.99657	-3.02349	103.99320	-2.01566
	CID	C3 H4 O1 S2	119.96981	-	-1.00727	72.02058	-
		C3 H2 O1*	54.01002	-	-1.00727	137.98037	-

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

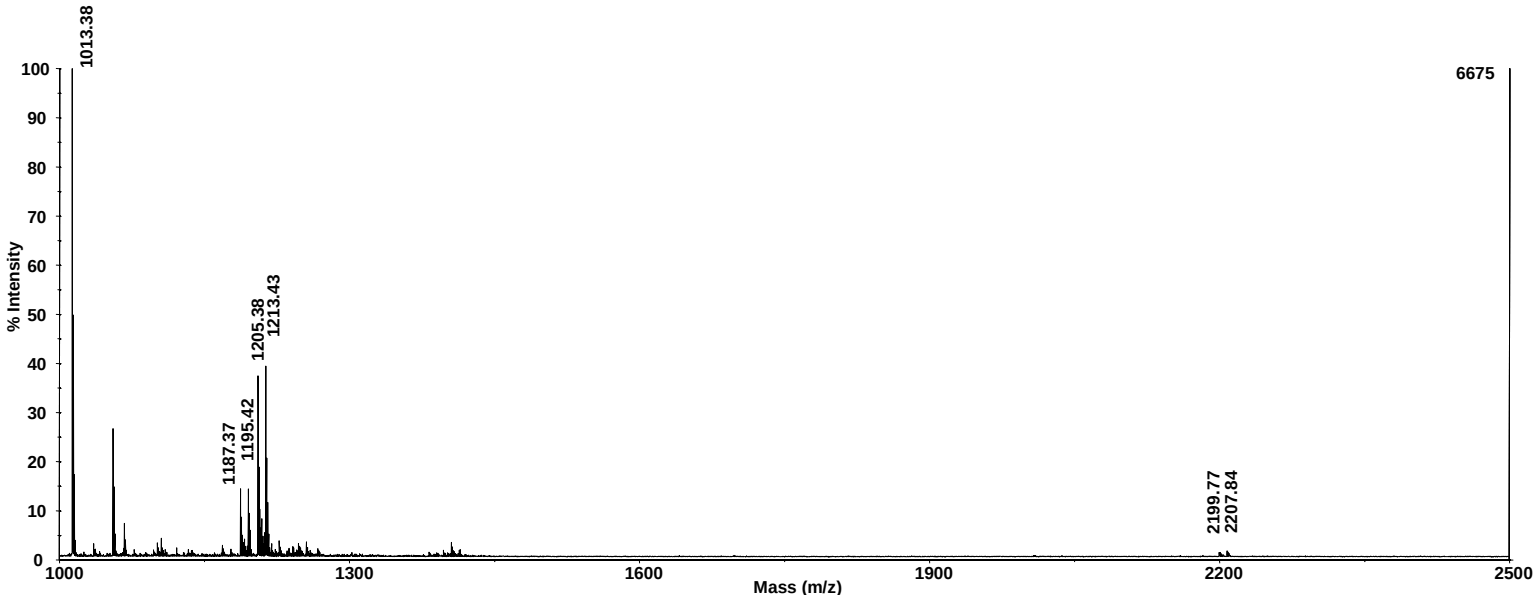


Figure 1. Mass spectrum of reaction products of the FLAG peptide modified with DTSSP-H8/D8.

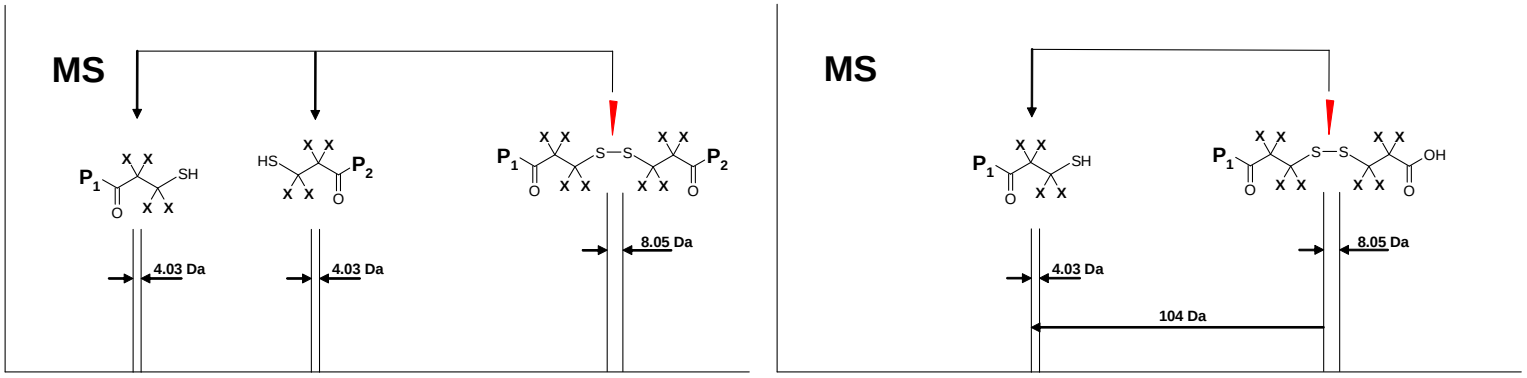


Figure 2. Scheme of chemical cleavage of DTSSP-H8/D8 inter-peptide (left panel) and dead-end (right panel) crosslinks.

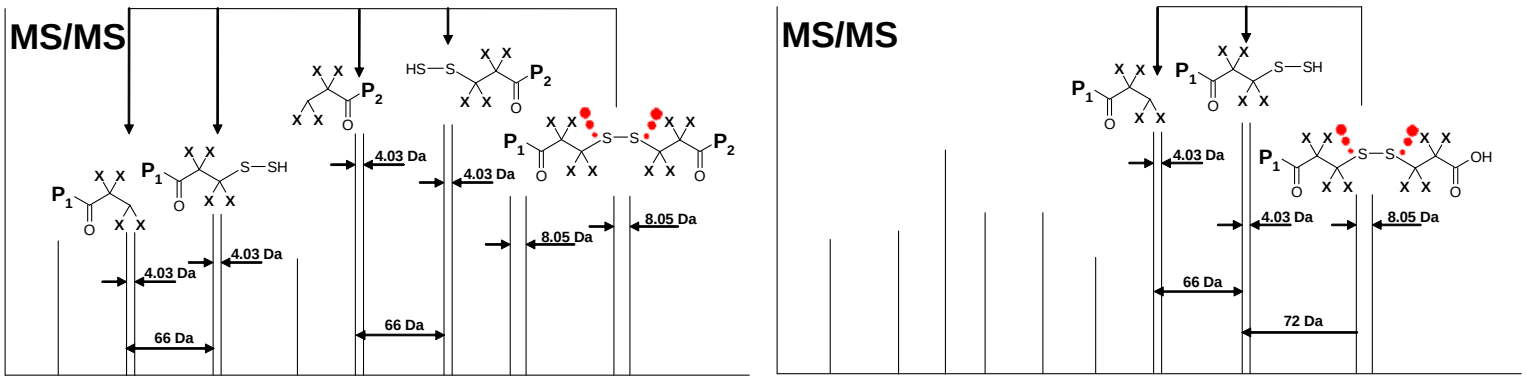


Figure 3. Scheme of CID cleavage of DTSSP-H8/D8 inter-peptide (left panel) and dead-end (right panel) crosslinks.

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

References:

1. Bennett KL, Kussmann M, Björk P, Godzwon M, Mikkelsen M, Sørensen P, Roepstorff P.
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A collection of novel isotopically-coded crosslinkers for structural proteomics.
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3. King GJ, Jones A, Kobe B, Huber T, Mouradov D, Hume DA, Ross IL.
Identification of disulfide-containing chemical cross-links in proteins using MALDI-TOF/TOF-mass spectrometry.
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