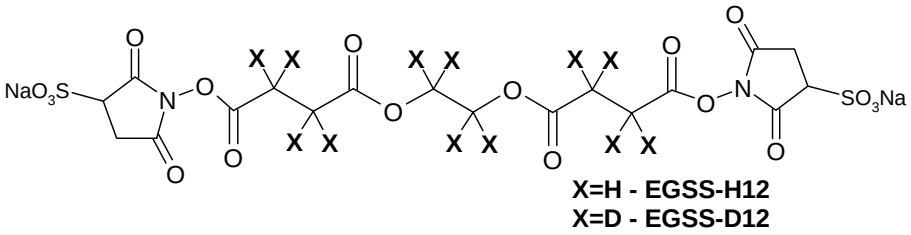
EGSS-H12/D12

Product Information



EGSS-H12/D12
EthyleneGlycol-di-SulfoSuccinimidylsuccinate
12 x 1 mg of 1:1 molar ratio mixture of EGSS-H12 and EGSS-D12
Cat. Number: 003SS
Formula: $C_{18}H_{18}N_2Na_2O_{18}S_2$ / $C_{18}D_{12}H_6N_2Na_2O_{18}S_2$

Molecular Weight: 660 / 672

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

EGSS-H12/D12 is a water-soluble, homobifunctional, isotopically-coded cleavable crosslinker EthyleneGlycol-di-SulfoSuccinimidylsuccinate. Light (H12) and heavy (D12) forms of the reagent differ by 12 deuterium atoms in heavy form instead of 12 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 EGSS-H12/D12 will manifest in mass spectra as doublets of peaks of equal intensity corresponding to light (H12) and heavy (D12) forms of the reagent separated by 12.07573 Da divided by charge state (12.07 for +1, 6.04 for +2, 4.03 for +3 etc.).

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. As EGSS is base-cleavable, high pH buffers or solutions tended to raise pH during incubation, like ammonium bicarbonate, should be avoided for the overnight trypsin digest. EGSS is water-soluble and stock solutions may be prepared in water. To make 50 mM stock solution of the EGSS-H12/D12, add 30 μl water to the pre-weigh tube containing 1 mg of the reagent.

To calculate masses of peptide crosslinks use following formulas:
$[M_{12}+H]^+ = [M_1+H]^+ + [M_2+H]^+ + 225.03991$
$[M_1OH+H]^+ = [M_1+H]^+ + 244.05830$
$[M_1i+H]^+ = [M_1+H]^+ + 226.04774$
$[M_1NH_2+H]^+ = [M_1+H]^+ + 243.07428$
, 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: C10 H10 O6; modification elemental composition for –OH dead-ends C10 H12 O7; modification elemental composition for $–NH_2$ dead-ends: C10 H12 N1 O6.

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 (H12) form of the reagent are: 1013 – free FLAG peptide; 1239 – intra-peptide crosslink; 1257 – dead-end crosslink; 2251 – inter-peptide crosslink.

EGSS-H12/D12 crosslinks can be cleaved by bases (Figure 2) (Ref. 1). 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. Cleavage can be conveniently achieved by incubation of the crosslinks with 1M NH_4OH for two hours at 25°C. Cleaved succinic moiety of the crosslinker attached to the amino group of the peptide cyclesizes into succinimidyl group (Figure 2), (Ref. 1). Milder conditions may result in incomplete cleavage of the crosslinker leading to the presence of additional possible products: succinylethyleneglycol (H8/D8) and succinate (H4/D4), (Table 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 DTSP crosslinks cleavage products.

Reagent	Cleavage	clrest el. comp.	Mclrest	Mclirest	Mcliploss	Mclohloss	Mcliloss
EGS	NH_4OH	C4 H2 O2	82.00548	164.01096	61.02895	162.05282	62.03678
		C4 H4 O3*	100.01604	200.03208	43.01838	144.04226	44.02621
		C6 H8 O4*	144.04226	226.04774	25.00728	100.01604	0
		-*		244.05830	-1.00727		-18.03437
		-*			-19.01839		

*- incomplete cleavage.

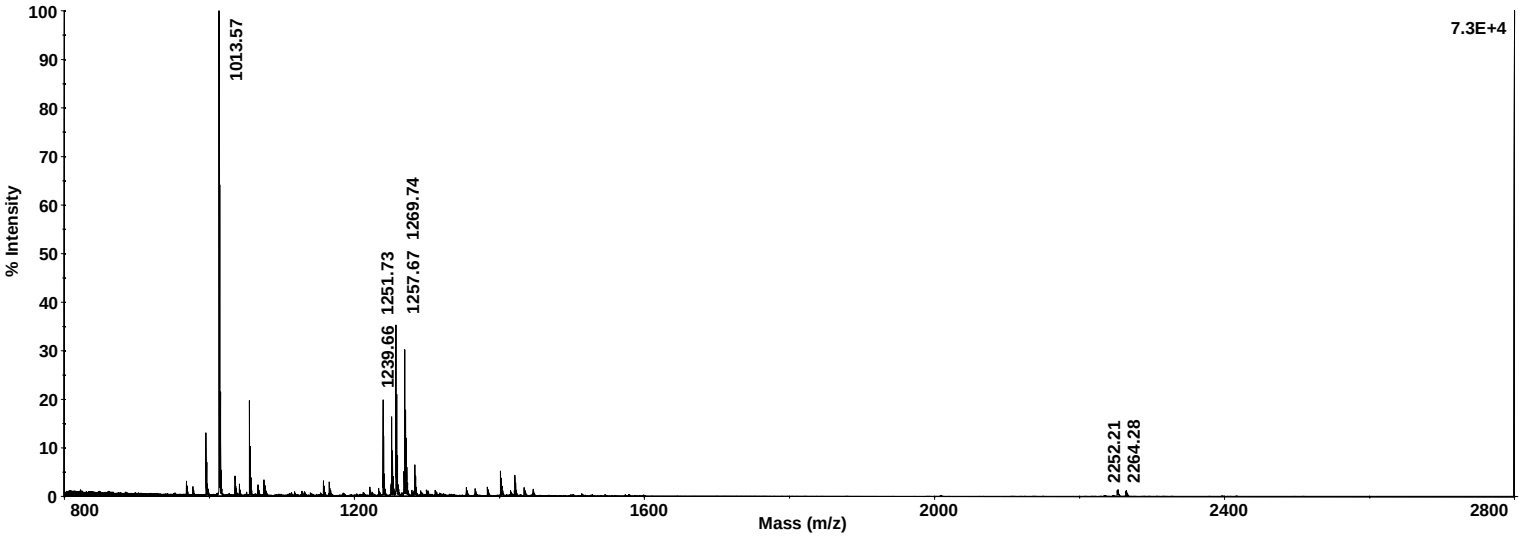
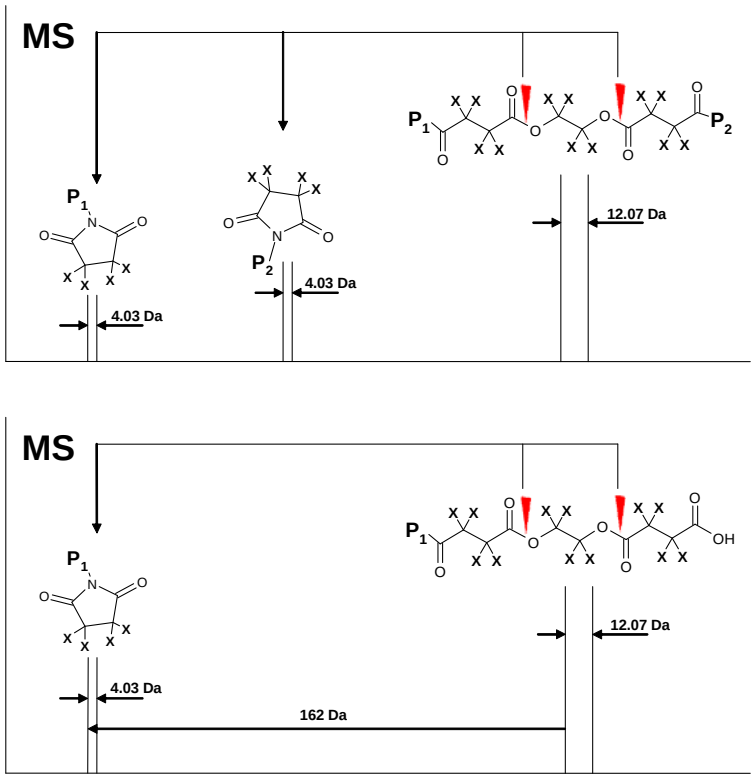


Figure 1. Mass spectrum of reaction products FLAG peptide modified with EGSS-H12/D12.



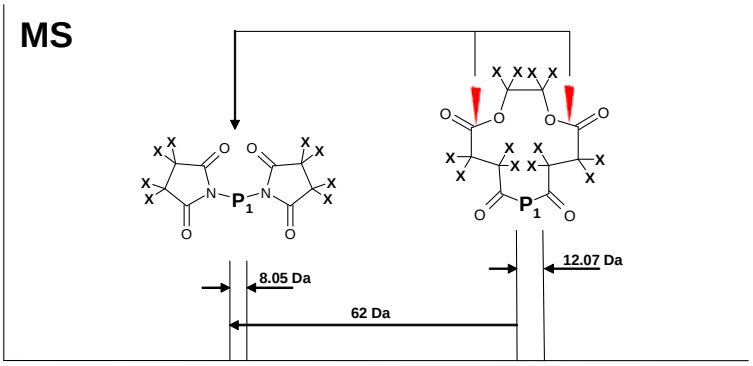


Figure 2. Scheme of chemical cleavage of EGSS-H12/D12 inter-peptide (top), dead-end (middle) and intra-peptide (bottom) crosslinks.

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

References:

1. Petrotchenko EV, Olkhovik VK, Borchers CH.
Isotopically coded cleavable cross-linker for studying protein-protein interaction and protein complexes.
Mol Cell Proteomics. 2005 Aug;4(8):1167-79.

