

**EGS-H12/D12****Product Information**

EGS-H12/D12

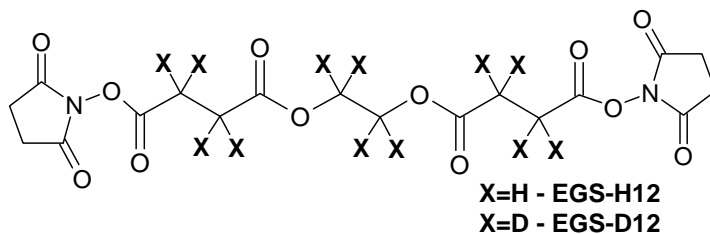
EthyleneGlycol-di-Succinimidylsuccinate

12 x 1 mg of 1:1 molar ratio mixture of EGS-H12 and EGS-D12

Cat. Number: 003S

Formula: C<sub>18</sub>H<sub>20</sub>N<sub>2</sub>O<sub>12</sub> / C<sub>18</sub>D<sub>12</sub>H<sub>8</sub>N<sub>2</sub>O<sub>12</sub>

Molecular Weight: 456 / 468

**Features:**

Isotopically-coded.

Membrane-permeable.

Chemically cleavable.

EGS-H12/D12 is a membrane-permeable, homobifunctional, isotopically-coded cleavable crosslinker EthyleneGlycol-di-Succinimidylsuccinate. 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 EGS-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-HydroxySuccinimide (NHS) esters react mainly with primary amino groups (-NH<sub>2</sub>) 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 EGS 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. EGS 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 50 mM stock solution of the EGS-H12/D12, add 43 μl DMSO 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<sub>1</sub>, M<sub>2</sub> - masses of free peptides; M<sub>12</sub> – mass of inter-peptide crosslink; M<sub>1</sub>OH – mass of dead-end crosslink; M<sub>1</sub>i – mass of intra-peptide crosslink; M<sub>1</sub>NH<sub>2</sub> – mass of dead-end amide (if reaction was quenched with ammonium salts).

MS-Bridge (<http://prospector.ucsf.edu>) bridge elemental composition: C<sub>10</sub> H<sub>10</sub> O<sub>6</sub>; modification elemental composition for –OH dead-ends C<sub>10</sub> H<sub>12</sub> O<sub>7</sub>; modification elemental composition for –NH<sub>2</sub> dead-ends: C<sub>10</sub> H<sub>12</sub> N<sub>1</sub> O<sub>6</sub>.

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.

EGS-H12/D12 crosslinks can be cleaved by bases (Figure 2) (Ref. 1). Cleaved moieties of the crosslink still isotopically labeled with H<sub>4</sub>/D<sub>4</sub> 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<sub>4</sub>OH for two hours at 25°C. Cleaved succinic moiety of the crosslinker attached to the amino group of the peptide cyclizes 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 (H<sub>8</sub>/D<sub>8</sub>) and succinate (H<sub>4</sub>/D<sub>4</sub>), (Table 1).

Cleaved crosslinks masses can be calculated using following formulas:

$$[M_{12}+H]^+ = [M_1cl+H]^+ + [M_2cl+H]^+ + Mcliploss$$

$$[M_1OH+H]^+ = [M_1cl+H]^+ + Mcl ohloss$$

$$[M_{1i}+H]^+ = [M_{1icl}+H]^+ + Mcl iloss$$

$$[M_1cl+H]^+ = [M_1+H]^+ + Mcl rest$$

$$[M_{1icl}+H]^+ = [M_1+H]^+ + Mcl i rest$$

, 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;  $M_{cliploss}$ ,  $M_{clohloss}$ ,  $M_{cliloss}$  – mass additions for cleaved inter-peptide, dead-end and intra-peptide crosslinks, correspondently;  $M_{clrest}$ ,  $M_{clirest}$  – 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.	$M_{clrest}$	$M_{clirest}$	$M_{cliploss}$	$M_{clohloss}$	$M_{cliloss}$
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
		.*			244.05830	-19.01839	

\*- incomplete cleavage.

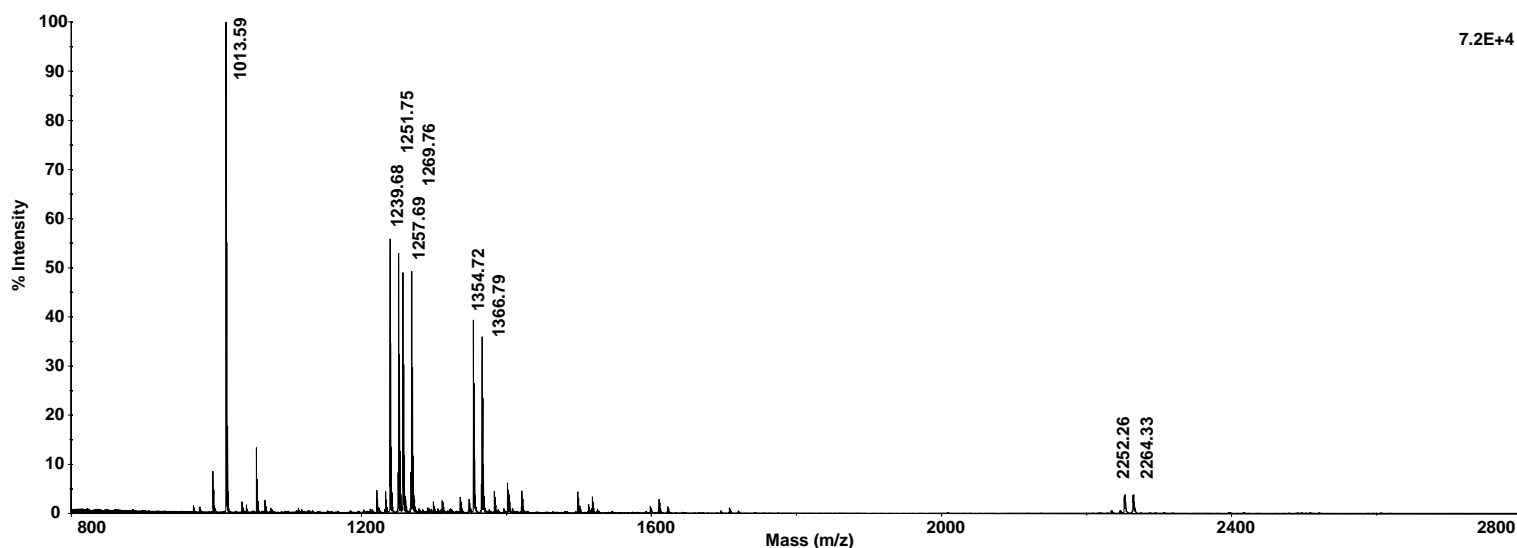
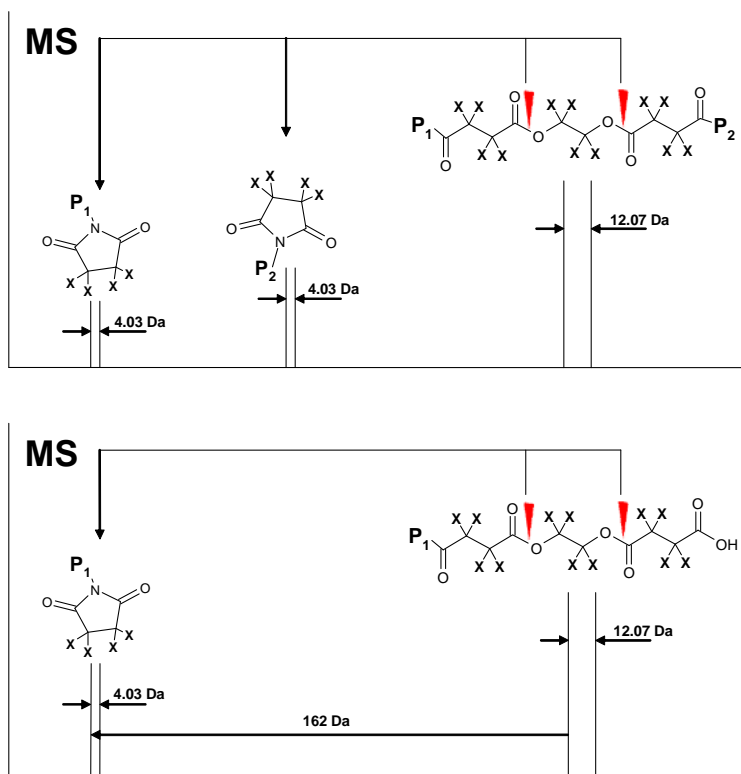


Figure 1. Mass spectrum of reaction products FLAG peptide modified with EGS-H12/D12.



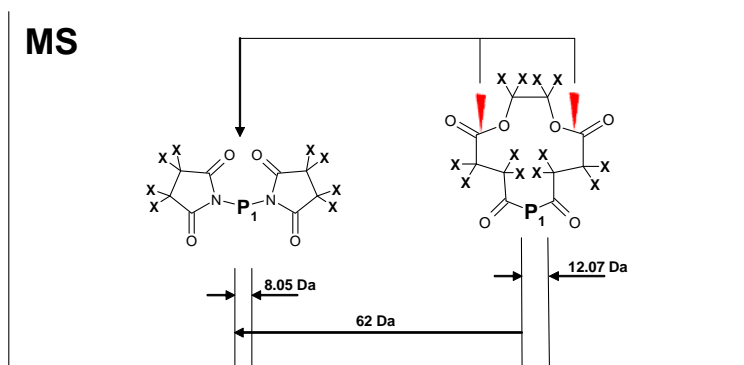


Figure 2. Scheme of chemical cleavage of EGS-H12/D12 inter-peptide (top), dead-end (middle) and intra-peptide (bottom) crosslinks.

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.