TP1

Product Information

Ac-TRTESTDIKRASSREADYLINKER

TP1
Test Peptide 1
12 x 1 nmol
Cat. Number: 008TP
Formula: C119 H201 N39 O44
Molecular Weight: 2880.46841

Features:
Can be converted to Lys-Lys canonical inter-peptide crosslink by enzyme treatment of intra-peptide crosslink.
High yield of crosslinking product.
Protected by acetylation N-terminus.
Optimized molecular weight for MALDI MS.

Test peptide Ac-TRTESTDIKRASSREADYLINKER is designed for characterizing crosslinked peptide products of amine-reactive crosslinking reagents. The peptide contains two lysine residues which can be efficiently crosslinked due to their spatial proximity and blocking by acetylation of the N-terminal amino group of the peptide. Trypsin digestion of the crosslinked peptide removes Ac-TR and ASSR peptides leading to the formation of the canonical Lys-Lys inter-peptide crosslink TESTDIKR-CL-EADYLINKER (Figure 1).

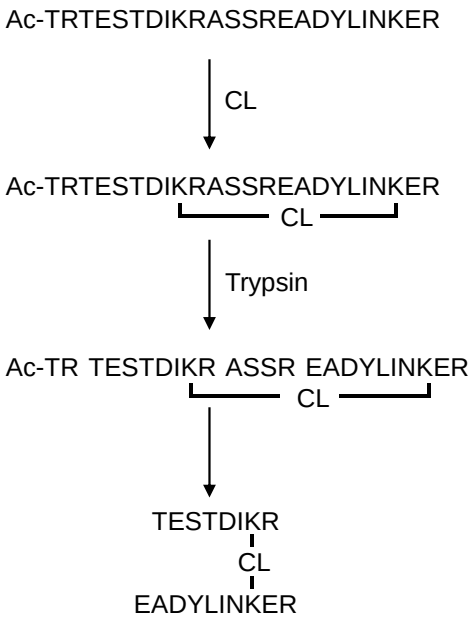

Figure 1. Scheme of producing of the canonical Lys-Lys inter-peptide crosslink from TP1.

Test peptide TP1 is supplied as a lyophilized material in 12 tubes each containing 1 nmol (2.8 μg) of peptide. Typical test crosslinking reaction of TP1 can be conducted in a similar way to the test crosslinking reagents reaction for FLAG peptide (http://www.creativemolecules.com/FLAG%20test%20reaction%20procedure.pdf). Lyophilized TP1 can be reconstituted in 20 μl of 100 mM triethylamine acetate buffer, pH 7.0 (Fluka) to give 50 μM solution of TP1. An equimolar amount of a crosslinking reagent (i.e. 2 μl of the 0.5 mM solution in water) can be added and the reaction mixture can be allowed to proceed at 25°C for 30 minutes. The reaction can be optionally quenched by addition 1 μl of freshly prepared 1M ammonium bicarbonate solution. 1 μg of trypsin (Promega) can be added and the mixture can be incubated for 3 hours at 37°C. 0.1 μl of the reaction mixture can be spotted on MALDI plate, dried, overlaid with 0.1 μl of matrix solution (1 mg/ml a-cyanohydroxycinnamic acid in 0.1% trifluoroacetic acid 50 % acetonitrile), dried and analyzed by MALDI MS.

Test peptide TP1 can be used as a standard material for new crosslinking reagents as well as control sample for crosslinking experiments ensuring correct performance during all processing steps of the peptide crosslinks.

Example mass spectrum of the TP1 crosslinked with BS3-H12/D12 and digested with trypsin is shown in Figure 2. Major crosslinked products are represented by dead-end crosslinks TESTDIKR-BS3-OH, EADYLINKER-BS3-OH and inter-peptide crosslink TESTDIKR-BS3-EADYLINKER with corresponding masses of 1105, 1406, 2337 Da.

To calculate theoretical masses of peptide crosslinks use following formulas:
$[M_{ipCL}+H]^+ = 949.49490 + 1250.63754 + Mip$
$[M_{OHCL}+H]^+ = 949.49490 + MOH$
$[M_{OHCL}+H]^+ = 1250.63754 + MOH$
, where M_{ipCL} – mass of inter-peptide crosslink; M_{OHCL} – mass of dead-end crosslink; 949.49490 – mass of singly charged protonated free TESTDIKR peptide; 1250.63754 – mass of singly charged protonated free EADYLINKER peptide; Mip and MOH – mass additions of crosslinking reagents for intra-peptide and dead-end crosslinks respectively (can be found in Table 2 of http://www.creativemolecules.com/CL%20masses.pdf). Thus, for BS3 Mip and MOH mass additions are 137.06025 Da and 156.07864 Da respectively, then theoretical masses for dead-end crosslinks TESTDIKR-BS3-OH, EADYLINKER-BS3-OH and inter-peptide crosslink TESTDIKR-BS3-EADYLINKER will be correspondently: 949.49490 + 156.07864 = 1105.57354, 1250.63754 + 156.07864 = 1406.71618, 949.49490 + 1250.63754 + 137.06025 = 2337.19269.

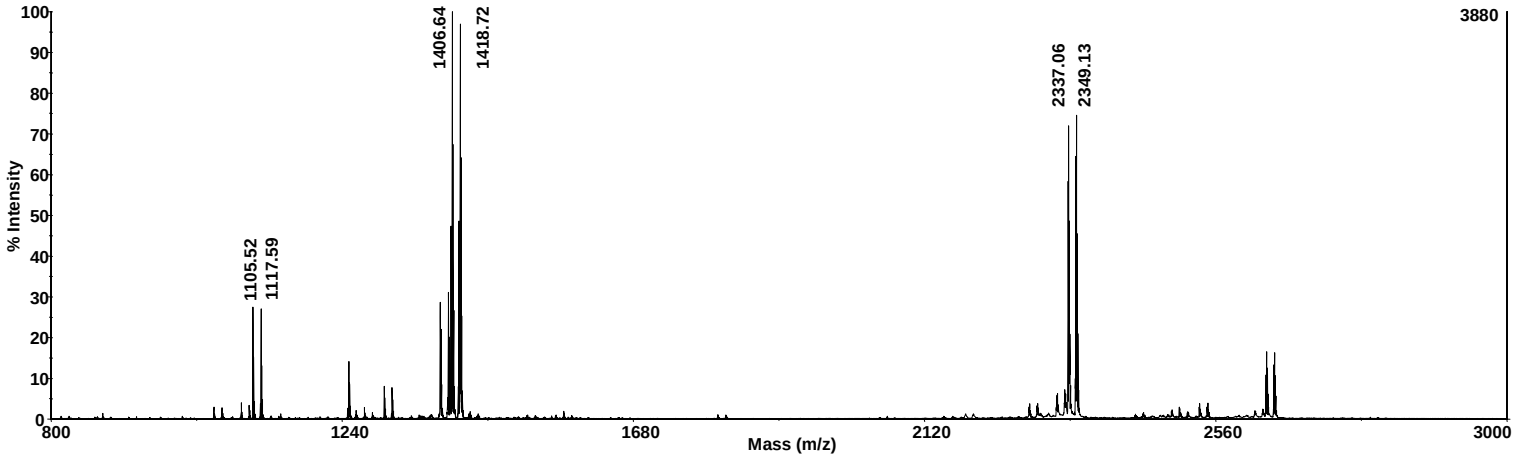

Figure 2. Mass spectrum of TP1 crosslinked with BS3-H12/D12 and digested with trypsin.

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