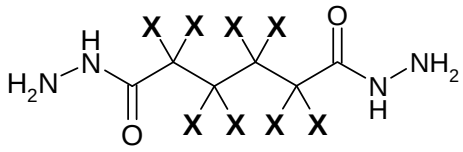
ADH-H8/D8



X=H - ADH-H8
X=D - ADH-D8

Product Information

ADH-H8/D8
Adipic acid DiHydrazide
12 x 1 mg of 1:1 molar ratio mixture of ADH-H8 and ADH-D8
Cat. Number: 019H
Formula: C6H14N4O2 / C6D8H6N4O2
Molecular Weight: 174 / 182

Features:
Isotopically-coded.
COOH-COOH crosslinking.

ADH-H8/D8 is an isotopically-coded Adipic acid 1,6-DiHydrazide which can form crosslinks between carboxy-groups when used together with carboxy-group activating reagents such as EDC or DMTMM [1,2]. 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 ADH-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.).

Dihydrazides will react with activated carboxy-groups (-COOH) in pH 5-7 buffers to form stable amide bonds. Therefore, amine- or carboxyl-containing buffers (Tris, Glycine, acetate, ammonium salts, etc.) should be avoided for crosslinking reaction. ADH is water-soluble and stock solutions can be prepared in water or buffer and then added to the aqueous reaction mixture. To make 250 mM stock solution of the ADH-H8/D8, add 22 µl H_2O to the pre-weigh tube containing 1 mg of the reagent.

To calculate masses of peptide crosslinks use following formulas:
$[M_1\text{-ADH-}M_2+H]^+ = [M_1+H]^+ + [M_2+H]^+ + 137.08217$
$[M_1\text{-ADH}+H]^+ = [M_1+H]^+ + 156.10056$
$[M_1\text{=ADH}+H]^+ = [M_1+H]^+ + 138.09000$
, where M_1, M_2 - masses of free peptides; M_1-ADH-M_2 – mass of inter-peptide crosslink; M_1-ADH – mass of dead-end crosslink; M_1=ADH – mass of intra-peptide crosslink.
Elemental composition of additions are C6 H9 N4, C6 H12 N4 O1 and C6 H10 N4 for inter-peptide, dead-end and intra-peptide crosslinks, respectively.

Typical MALDI mass spectrum of the test reaction with Nα-Acetyl-Arginine is shown in Figure 1. 20 µl reaction mixture containing 25 mM ADH-H8/D8, 50 mM Ac-Arg (Sigma) and 50 mM DMTMM (Sigma) in water was incubated overnight at 25°C and analyzed by MALDI MS. Masses of the reaction products for the light (H8) form of the reagent are: 217 – free Ac-Arg; 241 – DMTMM; 373 – dead-end Ac-Arg crosslink; 571 – inter-Ac-Arg crosslink. Masses 512 and 453 correspond to apparent loss of acetyl amino groups under MALDI conditions.

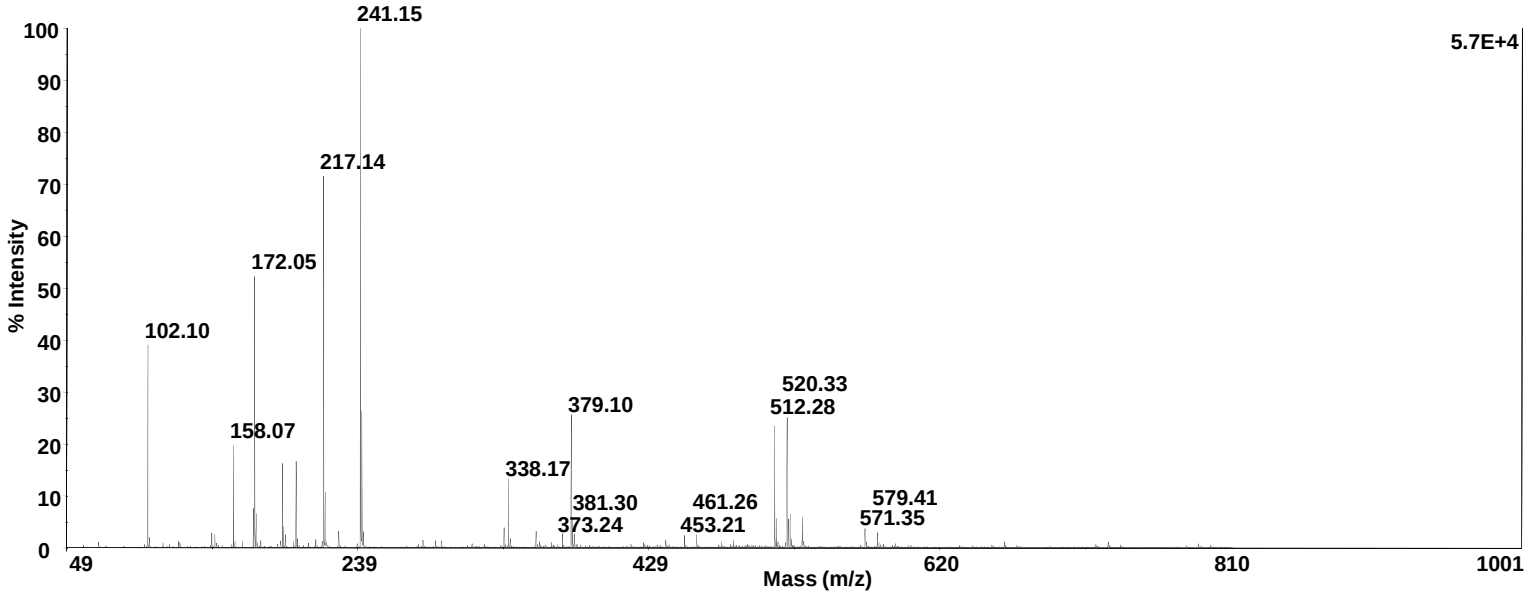


Figure 1. Mass spectrum of reaction products of Ac-Arg crosslinked with ADH-H8/D8 and DMTMM.

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

References:

1. Chemical cross-linking/mass spectrometry targeting acidic residues in proteins and protein complexes.
Leitner A, Joachimiak LA, Unverdorben P, Walzthoeni T, Frydman J, Förster F, Aebersold R.
Proc Natl Acad Sci U S A. 2014; 111(26):9455-60.

2. Intra-molecular cross-linking of acidic residues for protein structure studies.
Novak P, Kruppa GH.
Eur J Mass Spectrom (Chichester, Eng). 2008; 14(6):355-65.