

Crosslinking reagents test reaction procedure

Test reaction is crosslinking 50 μ M FLAG (DYKDDDDK) peptide (Sigma) with equimolar amount of crosslinking reagent for 30 minutes at 25°C.

The following procedure is used for test reaction of crosslinking reagents with FLAG peptide.

1 mg of crosslinking reagent (one tube) is reconstituted in appropriate amount (Table 1) of DMSO or water to give 50 mM stock solution. Stock solution is diluted then 100 times by adding 1 μ l to the 100 μ l of water to give 0.5 mM solution of the crosslinking reagent. 1 μ l of the resulting solution is added to 10 μ l of 50 μ M FLAG solution in 100 mM triethylamine acetate buffer, pH 7.0 (Fluka). Reaction mixture is incubated at 25°C 30 minutes. 0.1 μ l of the reaction mixture is spotted on MALDI plate, dried, overlaid with 0.1 μ l of matrix solution (1 mg/ml *o*-cyanohydroxycinnamic acid in 0.1% trifluoroacetic acid 50 % acetonitrile), dried and analyzed by MALDI MS on ABI 4800 TOF-TOF mass spectrometer.

Table 1. Volumes of solvent required to reconstitute 1 mg of the reagent to the 50 mM stock solution.

Cat. Number	Reagent	Mean M.W.	Volume, μ l
001S	DSS-H12/D12	374	53
001SS	BS3- H12/D12	578	35
002S	DTSP-H8/D8	408	49
002SS	DTSSP- H8/D8	612	33
003S	EGS- H12/D12	462	43
003SS	EGSS- H12/D12	666	30
004S	BiPS- H8/D8	598	33
004SS	BiPSS- H8/D8	802	25
005S	DNBDPS-H8/D8	574	35
005SS	DNBDPSS-H8/D8	778	26
006S	TEABS-H12/D12	889	22
006SS	TEABSS-H12/D12	1093	18

Reaction products of isotopically-coded reagents manifest in mass spectra as doublets of peaks of equal intensity corresponding to light (H) and heavy (D) forms of the reagent separated by 12.07573 Da (DSS, BS3, EGS, EGSS, TEABS, TEABSS) or 8.05325 Da (DTSP, DTSSP, BiPS, BiPSS, DNBDPS, DNBDPSS).

To calculate masses of peptide crosslinks for light (H) form of the reagent use following formulas:

$$[M_{11}+H]^+ = [M_1+H]^+ + [M_1+H]^+ + M_{ip}$$

$$[M_1OH+H]^+ = [M_1+H]^+ + MOH$$

$$[M_{1i}+H]^+ = [M_1+H]^+ + M_i$$

, where H – mass of proton; M_1 – mass of free neutral FLAG peptide (1012 Da); M_{11} – mass of inter-peptide crosslink; M_1OH – mass of dead-end crosslink; M_{1i} – mass of intra-peptide crosslink; M_{ip} , MOH , M_i – mass additions for inter-peptide, dead-end and intra-peptide crosslinks, correspondently (Table 2).

Table 2. Theoretical masses of FLAG test crosslinking reaction products, Da.

Cat. N.	Reagent	M_i	MOH	M_{ip}	$[FLAG+H]^+$	$[M_{1i}+H]^+$	$[M_1OH+H]^+$	$[M_{11}+H]^+$
001S	DSS-H12/D12	138.06808	156.07864	137.06025	1013.40581	1151.47389	1169.48445	2163.87187
001SS	BS3- H12/D12	138.06808	156.07864	137.06025	1013.40581	1151.47389	1169.48445	2163.87187
002S	DTSP-H8/D8	173.98093	191.99149	172.97310	1013.40581	1187.38674	1205.39730	2199.78472
002SS	DTSSP- H8/D8	173.98093	191.99149	172.97310	1013.40581	1187.38674	1205.39730	2199.78472
003S	EGS- H12/D12	226.04774	244.05830	225.03991	1013.40581	1239.45355	1257.46411	2251.85153
003SS	EGSS- H12/D12	226.04774	244.05830	225.03991	1013.40581	1239.45355	1257.46411	2251.85153
004S	BiPS- H8/D8	364.05515	382.06571	363.04732	1013.40581	1377.46096	1395.47152	2389.85894
004SS	BiPSS- H8/D8	364.05515	382.06571	363.04732	1013.40581	1377.46096	1395.47152	2389.85894
005S	DNBDPS-H8/D8	339.98238	357.99294	338.97455	1013.40581	1353.38819	1371.39875	2365.78617
005SS	DNBDPSS-H8/D8	339.98238	357.99294	338.97455	1013.40581	1353.38819	1371.39875	2365.78617
006S	TEABS-H12/D12	653.23668	671.24724	652.22885	1013.40581	1666.64249	1684.65305	2679.04047
006SS	TEABSS-H12/D12	653.23668	671.24724	652.22885	1013.40581	1666.64249	1684.65305	2679.04047

Test reaction is considered satisfactory when no major signals of unexpected reaction products are detected in spectra.