Lys-tag (Ktag) derivatization

(à la Ulf Hellman, LICR, Uppsala)

This derivatization modifies (the ε-amino groups of) lysine residues by the addition of the strongly basic 2-methoxy-4,5-dihydro-1H-imidazole, thereby enhancing the y-ion series of Lys-terminated tryptic peptides in MALDI-TOF PSD analysis. The modified peptide gains a mass of 68 amu.

At present, both 2-methoxy-4,5-dihydro-1H-imidazole and a tetradeuterated version of the compound are commercially available. A relatively simple synthesis protocol has been published by Peters and coworkers (Peters et al., Rapid Commun Mass Spectrom. 2001;15(24):2387-92).

Derivatization protocol

1. Dissolve one 0.5 mg aliquot of the derivatization agent (stored dry at -20 °C) in 10 μl of water, and transfer to a dried-down peptide sample. Vortex well/sonicate to dissolve the peptide.

2. Incubate at 55 °C for 2.5 h, and stop the reaction by adding 3.5 μl 10% TFA. Make sure that the pH is acidic before proceeding.

3. Clean up sample using STAGE, ZipTip or similar, and elute in small volume 60-70% ACN/0.1 % TFA.

Mix 1:1 with HCCA-solution (15 mg/ml in 50 % ACN, 49.9 % EtOH, 0.1 % TFA) and apply on MALDI target.