

N-terminal derivatization for *de novo* sequencing (Cotter Lab, 2003)

Addition of a negatively-charged sulfonate moiety on the amino terminus of peptides following digestion with trypsin results in the almost exclusive formation of y-series fragment ions from singly-protonated peptides. This provides an opportunity for de novo sequencing and for observing post-translational modifications.

1. All chemicals should be analytical grade. SPITC (4-sulfophenyl isothiocyanate), O-methyl isourea, sodium bicarbonate and ammonium bicarbonate are from Sigma (St. Louis). Bovine pancreas modified trypsin is from Roche Diagnostics Corp. (Indianapolis, IN).
2. Prepare the reagent solution by dissolving SPITC (10 mg/mL) in 20 mM NaHCO₃ (pH~9.0).
3. Carry out the sulfonation reaction in a 0.6 mL Eppendorf tube by mixing 9 μ L of reagent solution with 1 μ L of peptide solution (~10-100 pmol). After incubation for 30 min at 55° C, terminate the reaction by adding 1 μ L of 1% trifluoroacetic acid (TFA).
4. Load the sample onto a micropipette tip (C18 OMIX, Varian, Lake Forest, CA), wash with 3 x 10 μ L of 0.1% TFA, and follow by eluting with 10 μ L of 75% acetonitrile/o.1% TFA.
5. Take the solution to dryness using a SpeedVac and resuspend with 10 μ L of ddH₂O.
6. Analyze peptides using HPLC fractionation and tandem mass spectrometry.
7. *Guanidination.* Conversion of carboxy-terminal lysine residues (from tryptic digestion) to homoarginines prevents unwanted sulfonate tagging of these residues, increases their basicities and can improve the selective formation of y-series ions; and so the following procedure may be used on peptides prior to N-terminal sulfonation.
8. Prepare the reagent used for this reaction, O-methyl isourea, in ddH₂O to a concentration of 1 mg/ μ L.
9. React approximately 2 μ L of peptide (~100 pmol) with 1.5 μ L of the reagent solution and 5.5 μ L of ammonium hydroxide (30%) at 55° C for 30 min.
10. Terminate the reaction with 10 μ L of 10% TFA, and follow by purification using micropipette tips as described above.