

Determination of SUMOylation sites (Cotter Lab, 2005)

Unlike ubiquitin, SUMO proteins do not contain a convenient C-terminal cleavage site that can be used as a tag for identifying the SUMOylation site on a protein. The approach in this case uses two enzymes, chymotrypsin and trypsin, to provide a short SUMO modification sequence attached to tryptic peptides.

1. All chemicals should be of analytical grade or better. Sodium bicarbonate, ammonium bicarbonate (AmBic), trifluoroacetic acid (TFA) and proteomics grade trypsin are from Sigma (St. Louis). Sequencing grade bovine pancreas chymotrypsin is a salt-free lyophilizate from Roche Applied Science (Indianapolis, IN).
2. Digest SUMOylated proteins by adding sequencing grade chymotrypsin (1 μ L of 1 mg/mL solution in 1 mM HCl) in 1:50 enzyme:substrate ratio, and incubate the solution for 4 hours at room temperature (25° C).
3. Add proteomics grade trypsin (1:25, 2 μ L of 1 mg/mL solution in 1 mM HCl), and incubate overnight (approximately 15 hours) at 37° C.
4. Quench the digestion with the addition of 2 μ L of formic acid.
5. Evaporate to dryness and reconstitute with 5% acetonitrile (ACN) in water containing 0.1% TFA for analysis by LCMS or offline HPLC.
6. Obtain tandem mass spectra of peptide fractions. Incomplete digestion with chymotrypsin and the longer digestion time for trypsin is intended to produce an abundance of lysine or arginine-terminated peptides.
7. SUMOylated peptides are recognized by the presence of characteristic b' and y' ions from the branched sequence, in addition to the b and y ions from the peptide. These branched sequences are QEQTGG, QQQTGG, QQQTGG, and QQPTGG for SUMO-1, -2, -3 and -4, respectively.
8. For peptides containing SUMO-1,2 sites, prominent characteristic ions observed are b'₂-17 (m/z 240.12), b'₃-17 (368.12) and b'₄-17 (469.2). These can be observed in normal product ion mass spectra, but may also be used in precursor ion schemes.