

HYDROXYLAMINE CLEAVAGE OF Asn-Gly

1. 4 M guanidine HCl and hydroxylamine.
2. Buffer to pH 9 with 0.2 M NaCO₃. Will need to add ~ 16 volumes of NaCl₃ to reach pH 9 since solution is very acidic.
3. Add 5mM DTT, 1mM N-ethylmaleimide and 1x TXSWB to protein solution.
4. Mix equal volumes of guanidine-hydroxylamine solution with your protein. Final protein conc. should be 1-5 mg/ml.
5. Adjust pH to 9 if necessary with NaCO₃
6. Incubate 4-8 hours at 45(C.
7. Neutralize pH with acetic acid.
8. TCA precipitate protein.