Digestion protocol for single protein

This protocol for digestion of single protein the procedure follows.

1) Take 100 µg of the individual protein.
2) Add 100 µl of (100mM) Ammonium bicarbonate (ABC).
3) Add 20% of ACN (approx. 20 µl of ACN).
4) Incubate for 45 minutes at 60^{o}C.
5) **Reduction**: Add 5 µl of (50mM) Dithiothreitol (DTT) and incubate for 45 minutes at 60^{o}C (If protein do not have disulphide bonds, skip this step)
6) **Alkylation:** Add 5 µl of (50mM) Iodoacetamide (IAA) and incubate in dark at 45 minutes at room temperature (If protein do not have disulphide bonds, skip this step)
7) Add Trypsin in 1:50 ratio (Enzyme:Substrate) and incubate for 24 hours at 37^{o}C in shaking water bath.
8) Add 2 µl Trifluoroacetic acid (TFA) or Formic acid to stop the Trypsin activity.
9) Then subject the sample to speed vacuum to complete dryness.
10) Add 10 µl of 0.1 % TFA in water solution to reconstitute.
11) Then perform Zip-Tip to reduce the salt concentration from the sample.