In Solution Digestion

This protocol is useful when dealing with particularly small amounts of protein and/or less complex mixtures of protein, or highly abundant proteins.

As it is all done in the same eppendorf, certain factors (for example your protein concentration in µg/µl) must be obtained to ensure optimum activity of each reagent. Re-solubilising a pellet with 50mM Ammonium bicarbonate is preferable but you may need to use a small volume of 4-8M Urea if the pellet is more resistant. Once the proteins are in solution then the 4-8M Urea must be diluted back down to 1M in order for the typsin (or other digestive enzyme) to work. The smaller the total volume for this reaction, the better.

Reagents

- **100mM Iodoacetamide (IAA) in 25mM NH_4HCO_3**
- **45mM Dithiothreitol (DTT) in 25mM NH_4HCO_3**
- **1µg/µl Trypsin Gold (50mM acetic acid reconstituted)**

Before starting the protocol, please ensure that your sample mix is very near to pH 8 (or whichever pH is required for your enzyme of choice- please check the product protocol).

N.B: The volumes given in this protocol are applicable for 100µg protein concentration. Up/down scaling may be required.

1. To your protein mixture, add 5µl of 45mM DTT (in 25mM NH_4HCO_3, to a final working concentration of 10mM) and incubate at 50°C on a heated shaker for 15mins.

2. Cool slightly then add 5µl of 100mM IAA (diluted in 25mM NH_4HCO_3) and incubate in the dark at room temp for 15mins.

3. Add 1:100 enzyme to substrate (µg) ratio of trypsin. (N.B: other digestive enzymes may require a different enzyme: substrate ratio, check your information booklet if unsure of the dilution). Incubate overnight at 37°C.

An aliquot can be taken both at the beginning and end of the protocol (to run on a gel, see below) to ensure digestion was efficient and complete.

Un-digested Sample

Digested sample (12hours @37°C)

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