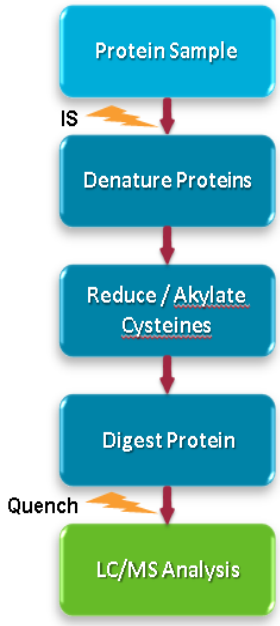
In-solution protein digestion for proteomic samples

Using the SCIEX Protein Preparation Kit

Protein digestion consists of a number of workflow steps with a variety of important reagents. Everything needed for a robust, reproducible digestion has been put together in this convenient kit.

1. The method begins with 5 µL of samples (35-350 µg total protein) per well for digestion.
2. Add 30 µL of Digestion Buffer (0.1 M TRIS, pH 8, 4mM CaCl2) to wells/vials
3. Add 2.5 µl of Denaturant (10% N-octyl-glucoside)
4. Add 5 µl of Reducing Reagent (50 mM of tris-(2-caroxyethyl)-phosphine))
    1. Cap and incubate at 60 °C for 1hr
    2. Spin plate/vials after incubation to bring any liquid down to the bottom before proceeding
5. Add 2.5 µL of Cysteine Blocking Reagent (200 mM of methyl methane-thiosulfonate)
    - Incubate at room temperature for 10 mins
6. Add 50 µL of Digestion Buffer to dilute sample before adding trypsin
7. Add 10 µL of Trypsin solution (dissolved in 0.1% formic acid)
    - Note – the trypsin amount can be adjusted depending on the total amount of protein being digested. Typically one uses a 1/10 to 1/20 ratio of trypsin / total protein. See Section 4 for an example calculation.
    - Cap and incubate off-deck for user desired # of hours at 37 °C (3 hours recommended)
    - Spin plate after incubation to bring any liquid down to the bottom before proceeding
8. Add 5 µL of Quench solution (user provided – 10% formic acid)



References

1. SCIEX Protein Preparation Kit (SCIEX P/N 4445247) and TPCK-treated trypsin (SCIEX P/N 4445250).
2. Automating protein digestion for reproducible proteomics. SCIEX technical note RUO-MKT-02-2364-A.

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