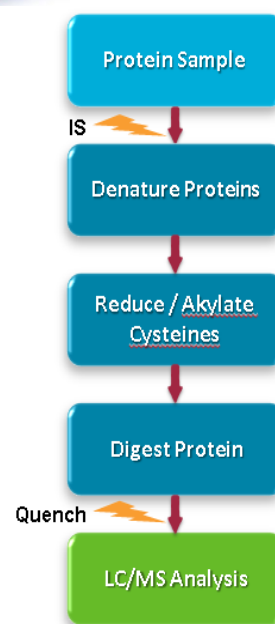


In-solution protein digestion for proteomic samples

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 μ L of samples (35-350 μ g total protein) per well for digestion.
2. Add 30 μ L of Digestion Buffer (0.1 M TRIS, pH 8, 4mM CaCl₂) to wells/vials
3. Add 2.5 μ L of Denaturant (10% N-octyl-glucoside)
4. Add 5 μ L of Reducing Reagent (50 mM of tris-(2-carboxyethyl)-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 μ L of Cysteine Blocking Reagent (200 mM of methyl methane-thiosulfonate)
 - Incubate at room temperature for 10 mins
6. Add 50 μ L of Digestion Buffer to dilute sample before adding trypsin
7. Add 10 μ L 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 μ L 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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