**IN-SOLUTION SAMPLE PREPARATION**

**The quality of sample extraction and preparation significantly impact MS results. For in-solution samples, strictly follow the following guidelines:**

* DO NOT wash any flasks, tubes, or glass plates (for sample preparation) with soap (or any polymeric detergent). Always, rinse your glassware with hot water and then an organic solvent like 70% ethanol or methanol.
* Freshly prepare all the buffers for MS analysis.
* **CRITICAL**: Mass spectrometry is highly sensitive to various contaminants, such as PEG (polyethylene glycol), keratin and various salts. Therefore, it is recommended to use powder free nitryl gloves and work in a dust- free environment.
* **CRITICAL**: The preferred MS compatible protein extraction buffer, ideal for optimum trypsin activity, is **50** **mM ammonium bicarbonate buffer**. If you had used any other buffer for washing or re-suspending the cell pellets (or tissues), **desalting** (or dialysis) and **buffer exchange** (using 50 mM ammonium bicarbonate) should be performed. It can be accomplished using a centrifugal filter with an appropriate molecular-weight-cut-off (MWCO) membrane such as Amicon Ultra 0.5 mL centrifugal filters having 3 kDa MWCO (P/N : UFC500324, Merk Millipore). **Please supply the protein sample in 50 mM ammonium bicarbonate buffer.**
* **CRITICAL**: The commonly used detergents are NOT compatible with MS. These include NP-40, Triton, CHAPS, SDS (sodium dodecyl sulfate) and LDS (Lithium dodecyl sulfate), Octyl glucoside, and octyl thioglucoside, sodium deoxycholate, lauryl maltoside, Brij-35, etc. If you have to use detergent for the cell lysis, use only MS-compatible detergents. A few MS-compatible detergents are listed below.
* RapiGest SF from Waters

(<https://www.waters.com/nextgen/in/en/shop/standards--reagents/186001861-rapigest-sf-1-mg--5-pk.html>)

* PPS Silent Surfactant from Agilent

(https://www.chem-agilent.com/pdf/strata/400500.pdf)

* Detergent-free buffer from Sigma

(<https://www.sigmaaldrich.com/IN/en/technical-documents/protocol/protein-biology/protein-lysis-and-extraction/extraction-from-tissue>)

* After desalting and buffer exchange, do protein quantification. The sample (protein) has to have a concentration of 1 mg/mL and 100 microlitres is required (100 micrograms in 100 microlitres). If the concentration of your sample is less than 1 mg/mL, let us know in advance.
* If you have samples to be compared (for relative protein quantification), all samples should be in uniform concentration and volume.
* We suggest sharing the protein isolation protocol (which you intend to follow) with the facility staff and getting their approval before proceeding with the sample preparation.
* Shipment: The samples need to be shipped in a frozen condition in dry ice or brought to RGCB in ice by hand.