**HUPO HPP MS Resource Pillar Cell Prep Initiative (CPI)**

Please provide as much of the following as possible in an MS Word or text document.

*Materials*

Consumables such as pipette tips, centrifuge tubes

 Source/brand, model/part number, size specifications, material composition

*Reagents*

Solvents, chemicals, kits

 Source/brand, purity, part number

*Solutions*

Composition/concentration, pH (when appropriate)

Preparation method, including chemical weights, solvent volumes, details about stock solutions and subsequent dilution, additional steps (such as sonication), considerations for storage (stability, frequency of preparation, container size/composition)

*Cells*

Details about the cell line, source, culture conditions, method for initial isolation

Cell counting method

Cell sorting or enrichment (when used), including collection solution

*Step-by-step protocol*

Cell handling

 Centrifugation

 Washing

 Supernatant removal

Cell lysis

 Lysis buffer

 Lysis conditions (time, temperature)

 Mechanical disruption (if used)

Protein quantitative analysis (when used)

 Details about use of any kits (not just “manufacturer’s instructions”)

 Reference standard

 Expected protein quantity per cell type

Reduction/alkylation (if used)

 Buffer

 Reagents

 Conditions (time, temperature, protection from light)

Proteolytic digestion

 Format (e.g., solution, in-gel, filter-aided, or on solid support)

 Buffer

Digestion conditions

 Enzyme-to-protein ratio

 Time, temperature

Sample cleanup (if used)

Sample handling prior to MS analysis

 Solvent removal

 Storage format (i.e., dry or in solution)

 Storage temperature

 Re-dissolving for injection

 Solvent composition

 Mechanical assistance (e.g., sonication, vortexing)

Mass spectrometry analysis and data processing

 Provide a *brief* description. Additional details may be requested in the future.

 Name/model/vendor of instrumentation

 Summary of analytical strategy

 Data processing pipeline

 Software

 Database(s) (with name/date and number of sequences)

 Key search parameters

*Results*

Protein quantity/concentration per representative sample (average ±sd for a set of biological replicates)

Peptide quantity after digestion per representative sample (average ±sd for a set of biological replicates)

Peptide quantity injected

Protein identification

 Criteria for acceptance of peptide assignments and protein identifications

 Total number of identified proteins

 Total number of assigned peptide spectrum matches

 Data table (when available)

 Excel spreadsheet preferred

 Numerical entries formatted to display an appropriate number of decimal places depending on the accuracy of the primary data

 Identified proteins

 Protein name

 Accession number

 Molecular weight

 Total number of spectra used for identification

 Number of unique peptides used for identification

 Percent sequence coverage