**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