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| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: Trypsin digestion of human serum as background matrix for targeted mass spectrometry analysis** | | | **Version #: 1.1** | **Author: Hui Zhang Laboratory – Johns Hopkins University** | | **Date: 05/08/2014** |  | |

**Purpose**

The purpose of this document is to describe the method for the enzymatic digestion of serum that will be used as the background matrix for targeted mass spectrometry-based analytical methods.

**Scope**

This document describes the detailed procedures for Protein extraction, Trypsin digestion, and Peptide desalting.

**Responsibilities**

It is the responsibility of person(s) performing this procedure to be familiar with laboratory safety procedures. The interpretation of results must be done by a person trained in the procedure and familiar with such interpretation.

**Equipment**

* Vacuum centrifuge
* Temperature-controlled shaking incubator
* Speed-Vac
* NanoDrop spectrophotometer

**Materials**

* Human Serum (Sigma-Aldrich; catalog # H4522)
* 1 g 6 cc C18 SPE cartridges (Waters); NOTE: typical binding capacity is 1-5% of the C18 bed weight

**Reagents**

* Deionized water
* Urea – Ultra Pure (Thermo Fisher Scientific)
* TCEP (Thermo Fisher Scientific)
* Iodoacetamide (Sigma-Aldrich)
* Optima LC/MS-grade water (Thermo Fisher Scientific)
* Bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific)
* Sequencing-grade modified trypsin (Promega)
* Trifluoroacetic acid (Sigma-Aldrich)
* Ammonium bicarbonate (Sigma-Aldrich)
* Formic acid (Sigma-Aldrich)
* LC/MS-grade water (Thermo Fisher Scientific)
* LC/MS-grade acetonitrile (Thermo Fisher Scientific)

**Solutions**

* Urea buffer: 8 M urea in 0.4 M NH4HCO3
* 10 mM TCEP
* 12 mM Iodoacetamide
* 50 % ACN/0.1 % TFA
* 60 % ACN/0.1 % TFA

**Procedure**

1. **Protein extraction and trypsin digestion**
   1. Dilute serum 1:10 with urea buffer (8 M urea, 0.8 M NH4HCO3, pH 8.0).
   2. Measure the protein concentration using the BCA protein assay kit.
   3. Reduce disulfide bonds by adding DTT to the sample at a final concentration of 10 mM. Incubate the sample at 37 °C for 1 h.
   4. Carbamidomethylate cysteine residues by adding Iodoacetamide at a final concentration of 12 mM. Incubate the sample at RT for 1 h in the dark.
   5. Dilute the sample 1:4 with deionized water to reduce the urea concentration to 2 M.
   6. Digest the protein with trypsin using an enzyme-to-substrate ratio of 1:50 (wt/wt) for 12 h at 37 °C with mild shaking.
   7. Add another aliquot of the same amount of trypsin used in step 6 and incubate overnight at 37 °C with mild shaking.
   8. Acidify the sample by the addition of trifluoroacetic acid to a final amount of 10 % (vol/vol) and vortex. Make sure that the pH of the solution is ≤3 using a pH indicator strip before desalting.
2. **Peptide de-salting**
   1. Activate the C18 SPE column with 12 mL of 100 % ACN.
   2. Condition the C18 SPE column with 9 mL of 50 % ACN/0.1 % TFA.
   3. Equilibrate the C18 SPE column with 20 mL of 0.1 % TFA.
   4. Load the sample onto the C18 SPE column.
   5. Wash the C18 SPE column with 20 mL of 0.1 % TFA.
   6. Elute peptides with 6 mL of 60 % ACN/0.1 % TFA and dry in Speed-Vac.

**Referenced Documents**

1. Sun S, Zhou JY, Yang W, Zhang H. Inhibition of protein carbamylation in urea solution using ammonium-containing buffers. Anal Biochem. 2014 Feb 1;446:76-81. doi: 10.1016/j.ab.2013.10.024. Epub 2013 Oct 23. PubMed PMID: 24161613; PubMed Central PMCID: PMC4072244.