High-throughput Multiplexed SISCAPA® Assay for Targeted Protein Quantitation by LC-MS/MS

Material and Method

**Introduction**

SISCAPA® (Spike-in Competitive Analysis by Peptide Array) is a high-throughput method for detection and quantification of proteins. This method involves the use of synthetic peptides as standard materials to be used in the quantitation of endogenous proteins. The SISCAPA® technology is based on the principle of competition between the synthetic peptides and their corresponding endogenous protein targets for a limited number of binding sites on a solid support. This competition is monitored using mass spectrometry (LC-MS/MS), allowing for the detection and quantification of proteins with high sensitivity and specificity.

**Material and Method (continued)**

**Sample preparation**

- **Sample volume**: 135 µL (containing 4 µL of human serum, internal standard peptides, appropriate amount of calibrator peptides, in 67.5 mM ammonium bicarbonate)
- **Sample tryptic digestion**
  - Add entire digested sample (~135 µL) from PCR plate to the 2mL 96-well NUNC plate containing washed beads.
- **Wash Beads (2X)**
  - 5. Add 500 µL of 1XPBS+0.03%CHAPS to each well, cap plate and incubate for 1 hour at room temp using the Vibrax VXR Basic at 900-1000rpm.
  - 6. Place the 2mL 96-well NUNC plate on the magnet to separate the beads from solution and remove the wash solvent.
  - 7. Wash wells containing beads with 600 µL of 1xPBS+0.03%CHAPS.
  - 8. Add 1000 µL of 1xPBS+0.03%CHAPS to the tube, cap and vortex gently.
  - 9. Place the 2mL 96-well NUNC plate on the magnet to separate the beads from solution and remove the wash solvent.
  - 10. Wash wells containing beads with 600 µL of 1xPBS+0.03%CHAPS a second time.
  - 11. Add 125 µL of 0.1% formic acid in water + 0.03% CHAPS to each well for peptide elution.
  - 12. Resuspend beads by vortexing vial > 30 sec while tilting and rotating vial until no material is settled on the bottom of the vial.

**Liquid chromatography**

- **System**: ZAB-Quad® ESI-MS/MS
- **Column Temp**: 40°C
- **Total Flow**: 0.3 mL/min
- **Gradient**: 0.8 min 0-20%B, 0.8 min 20-40%B, 0.4 min 40%B, 0.2 min 40-65%B, 0.3 min 65-95%B, 0.7 min 95%B, 0.5 min 95-0%B, 1 min 0%B
- **Column**: Agilent AdvanceBio® Peptide Map 2.1 x 100mm 2.7-micron 600Bar
- **Ion Source**: Turbo Spray
- **Scan Type**: MRM
- **Wash 1**: 60/30/10 acetonitrile/isopropanol/acetone
- **Wash 2**: 75/25 water/acetonitrile with 1% formic acid
- **MPA**: water with 0.1% formic acid

**Representative chromatograms for Peptide 3**

**Conclusion**

The SISCAPA® technology offers a high-throughput method for the detection and quantification of proteins, allowing for the analysis of large sample sets with high accuracy and precision. This technology is particularly useful in research and clinical settings where the quantification of multiple proteins is required. Further studies are needed to validate the method for different sample types and conditions, and to optimize the parameters for specific applications.

**References**