

Interleukin Analysis by LCMS

Introduction

Cytokines are important biomarkers in the evaluation of the pathogenesis of diseases [1]. These small signaling proteins play a central role in cell survival and cellular inflammation. Notable signaling proteins interleukin-6 (IL-6) and interleukin-11 (IL-11), which both have well defined pro-inflammatory and anti-inflammatory functions [2]. The average circulating concentration of cytokines within an organism is low, typically in the pg/mL range. This circulating concentration, in conjunction the small size of cytokines, makes signaling proteins like IL-6 and IL-11 historically difficult to locate with the use of commercially available assays kits [1].

Why use Spectrus' Interleukin Serum Analysis?

Spectrus scientists have developed a viable method for detecting peptides of IL-6 and IL-11 cytokines in human serum using LC-MS analysis. Spectrus' serum analysis is able to reproducibly detect IL-6 peptides to 0.65 pg/ μ L and IL-11 peptides to 9.65 pg/ μ L. In serum, the detection of these peptides shows good sensitivity and reproducibility.

Methods in Brief

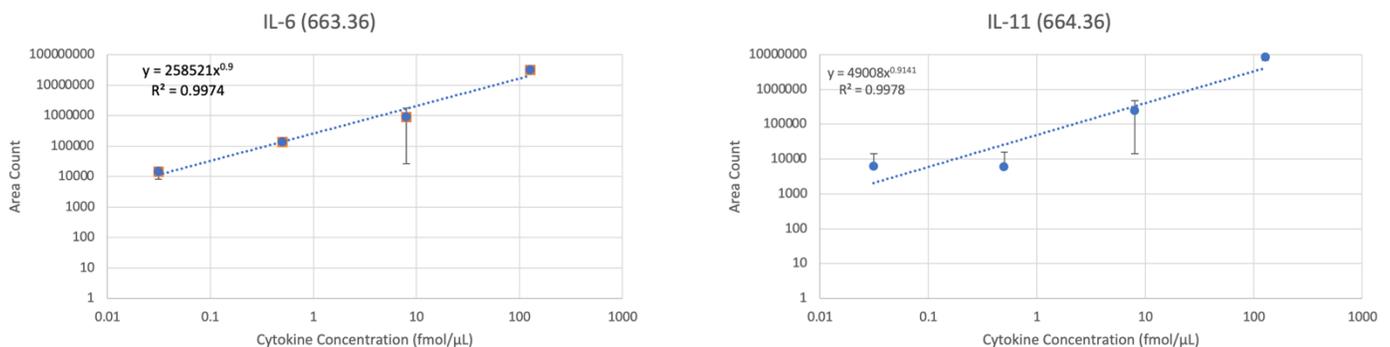
Intact mixture of cytokines and serum will follow the denaturation-reduction-alkylation-trypsin digest procedure and then be analyzed via LC-MS.

Proof of Concept

Spectrus scientists began the development of interleukin serum analysis by initially digesting a cytokine mixture for analysis by LC-MS. Peptides of IL-6 and IL-11 were quickly identified with good sensitivity and reproducibility were detected via this method of analysis. Similarly, good sensitivity and reproducibility was identified when a digest of cytokines was spiked into a serum digest and analyzed via LC-MS.

Next, an intact mixture of cytokine and serum was evaluated. Following the denaturation-reduction-alkylation-trypsin digest procedure, the resultant mixture was analyzed via LC-MS. Similar to previous results, peptides of IL-6 and IL-11 were identified with good sensitivity and reproducibility. (Figure 1)

Figure 1. Peptides of IL-6 and IL-11 illustrating linearity and detection of low levels of peptide



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Summary of Findings

As seen in Figure 1, IL-6 peptide 663.36 is linear from 0.03125 fmol/ μ L to 128 fmol/ μ L. The dynamic range is 4096, and the lowest detectable concentration is 0.65pg/ μ L. IL-11 peptide 664.36 is linear from 0.5 fmol/ μ L to 128 fmol/ μ L. The dynamic range is 256 with the lowest detectable concentration of 9.65 pg/ μ L.

Why Spectrus is the right is choice for Interleukin Serum Analysis

Spectrus scientists have developed a unique method for determining the concentration of peptides of IL-6 and IL-11 in serum with good sensitivity and reproducibility.

Bibliography

- [1] N. Aziz, "Measurement of Circulating Cytokines and Immune-Activation Markers by Multiplex Technology in the Clinical Setting: What Are We Really Measuring?," *Forum on Immunopathological Diseases and Therapeutics*, vol. 6, no. 1-2, pp. 19-22, 2015.
- [2] J. Lokau, M. Agthe and C. Garbers, "Generation of Soluble Interleukin-11 and Interleukin-6 Receptors: A Crucial Function for Proteases during Inflammation," *Mediators of Inflammation*, p. 1785021, 2016.