# IL-6 ELISA Analysis

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**Tradename:** Phyto-Biotics Perilla®

**Code:** 40600

**CAS #:** 90082-61-4 & 56-81-5 & 57-55-6 & 1686112-36-6 (or) 68333-16-4

**Test Request Form #:** 404

**Sponsor:** *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
**Study Director:** *Erica Segura*
**Principle Investigator:** *Meghan Darley*

**Test Performed:**
Interleukin (IL)-6 Enzyme-Linked Immunosorbent Assay (ELISA)

## Introduction

Interleukin-6 is a proinflammatory cytokine known to play an active role in inflammation, immunology, bone metabolism, reproduction, arthritis, neoplasia, and aging. IL-6 signals through the nuclear factor-kappa B (NF-κB) pathway that results in the transcription of inflammatory mediators, including matrix metalloproteinase-1 (MMP-1). MMP's are responsible for breaking down the extracellular matrix and collagen in the skin leading to wrinkles, fine lines, and loss of skin elasticity. Reducing the level of IL-6 and other inflammatory mediators is believed to slow down degradation of the skin matrix and, possibly, stimulate its replenishment.

Interleukin-6 ELISA was conducted to assess the changes in IL-6 levels in **Phyto-Biotics Perilla®**-treated *in vitro* cultured human dermal fibroblasts.

## Assay Principle

This ELISA utilizes a colorimetric reaction employing antibodies with antigen specificity to human IL-6. Monoclonal antibodies specific for IL-6 epitopes are coated on a microtiter plate. In positive samples, IL-6 will bind to these antibodies and are tagged a second time with another IL-6-specific antibody labeled with horseradish peroxidase (HRP). The addition of the chromagen solution, containing 3,3',5,5'-tetramethylbenzidine, provides the colorimetric reaction with HRP that is quantitated through optical density (OD) readings on a microplate spectrometer. The standard curve provides a reference from the OD readings for the amount of collagen in each sample.

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## Materials

| | |
|---|---|
| **A. Kit:** | IL-6 ELISA Kit (Biosource; KAC1261) |
| **B. Incubation Conditions:** | 37°C at 5% $CO_2$ and 95% relative humidity (RH) |
| **C. Equipment:** | Forma humidified incubator; ESCO biosafety laminar flow hood; Microplate Reader; Pipettes |
| **D. Cell Line:** | Normal Human Dermal Fibroblasts (NHDF) (Lonza; CC-2511) |
| **E. Media/Buffers:** | Dulbecco's Modified Eagle Medium (DMEM); Penicillin-Streptomycin (50U-50mg/mL); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS); Amphotericin (45pg/mL) |
| **F. Culture Plate:** | Falcon flat bottom 12-well tissue culture treated plates |
| **G. Reagents:** | Lipopolysaccharide (LPS) (1µg/mL) |
| **H. Other:** | Sterile disposable pipette tips; wash bottles |

## Methods

Human dermal fibroblasts were seeded into 12-well tissue culture plates and allowed to grow to confluency in complete DMEM. 1%, 0.1%, 0.01% concentrations of **Phyto-Biotics Perilla®** were added to complete DMEM containing 1µg/mL LPS and incubated with fibroblasts for 24 hours. Complete media containing 1µg/mL LPS was used as the positive controls and complete DMEM was used a negative control.

Standards were prepared in concentrations ranging from 2476pg/mL to 0pg/mL. 50µL of Solution B was added to wells for standards and assay controls and 50µL of Solution A was added to experiment wells. 100µL of standards, controls, and samples were added to appropriate wells. After a one hour incubation at room temperature and washing, 50µL Solution A and 100µL anti-IL-6 conjugate was added to all wells. Following a one hour incubation and washing, 100 µL chromagen solution was added for the colorimetric reaction. One-hundred µL stop solution was added to stop the reaction after 15 minutes. The optical density was read at 450nm on the Synergy HT Microplate Reader.

A standard curve was created by reducing the data and generating a linear curve fit. The IL-6 concentration of **Phyto-Biotics Perilla®** treated-fibroblasts was determined by extrapolation from the standard curve and expressed in pg/mL.

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## Results

The data obtained from this study met criteria for a valid assay and the positive and negative controls performed as anticipated.

**Phyto-Biotics Perilla®,** at concentrations of 1%, 0.1%, and 0.01% was able to decrease IL-6 production compared to our positive control.

IL-6 levels are expressed by the following formula:

$$= Average_{IL-6\ Concentrations} \times Dilution\ Factor$$

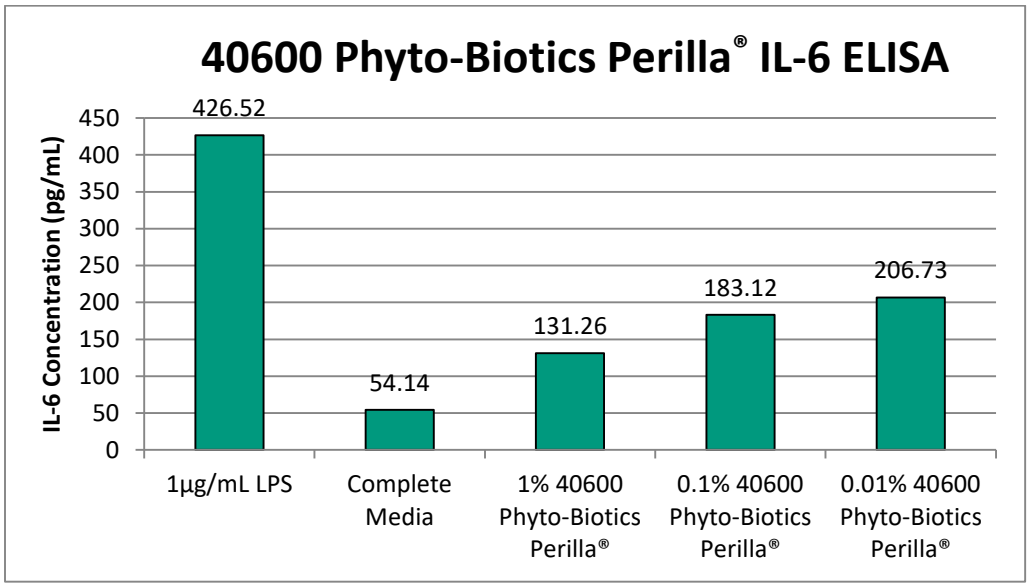


**Figure 1: Phyto-Biotics Perilla®**-treated fibroblasts IL-6 concentrations

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IL-6 production percent decrease is calculated by the following formula:

$$= \frac{Positive\ Control_{\ Avg.Concentration} - Sample_{\ Avg.Concentration}}{Positive\ Control_{\ Avg.Concentration}} \times 100$$

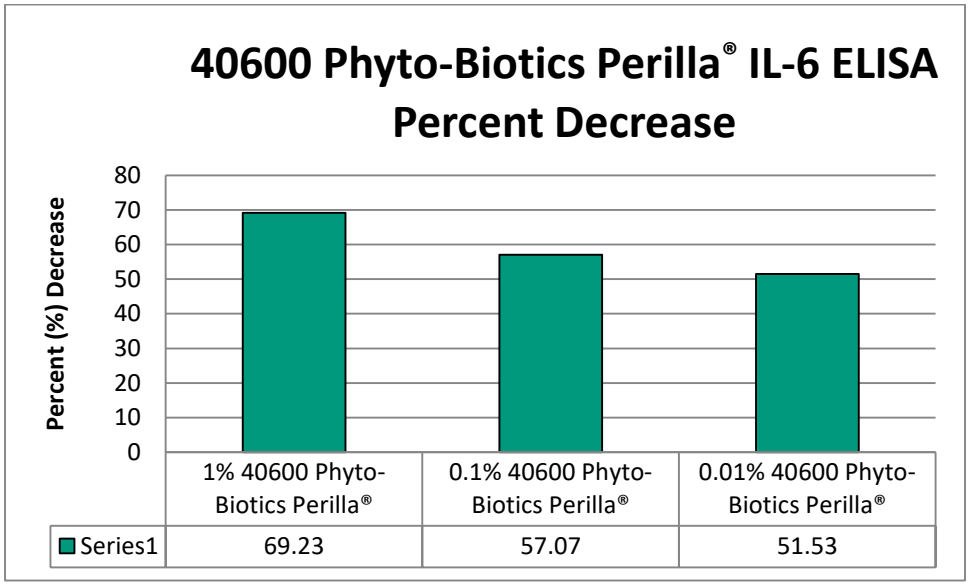


**Figure 2:** Percent decrease in IL-6 production compared to positive control

## Discussion

As shown in figure 1, **Phyto-Biotics Perilla®** exhibited anti-inflammatory effects on LPS-treated fibroblasts. As expected, the changes in IL-6 production using **Phyto-Biotics Perilla®** appears to be dose dependent. This decrease in IL-6 production indicates a reduced inflammatory environment which could decrease the signs of aging and reduce the formation of fine lines and wrinkles. For these reasons, we can assume **Phyto-Biotics Perilla®** is suitable for cosmetic applications designed to provide soothing and anti-aging properties.