



## IL-6 ELISA Analysis

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**Tradename:** AC Vegetable Ceramides G

**Code:** 16558G

**CAS #:** 56-81-5 & 308067-30-3

**Test Request Form #:** 6535

**Lot #:** 74759P

**Sponsor:** *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*

**Study Director:** *Maureen Danaher*

**Principle Investigator:** *Jennifer Goodman*

**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- $\kappa$ B) 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 **AC Vegetable Ceramides G** - 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 IL-6 in each sample.

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## Materials

- |                           |   |
|---------------------------|---|
| A. Kit:                   | Human IL-6 ELISA Kit (Thermo Scientific; EH2IL6)  |
| B. Incubation Conditions: | 37°C at 5% CO <sub>2</sub> 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 (HDFa) (ATCC; PCS-201-121)  |
| 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); Dexamethasone (10µM)   |
| 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 **AC Vegetable Ceramides G** were added to complete DMEM containing 1µg/mL LPS and incubated with fibroblasts for 72 hours. Complete media containing 1µg/mL LPS was used to create an inflammatory environment and dexamethasone (DEX) in the presence of LPS was used as a positive control to quell inflammation.

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 **AC Vegetable Ceramides G** treated-fibroblasts was determined by extrapolation from the standard curve and expressed in pg/mL.

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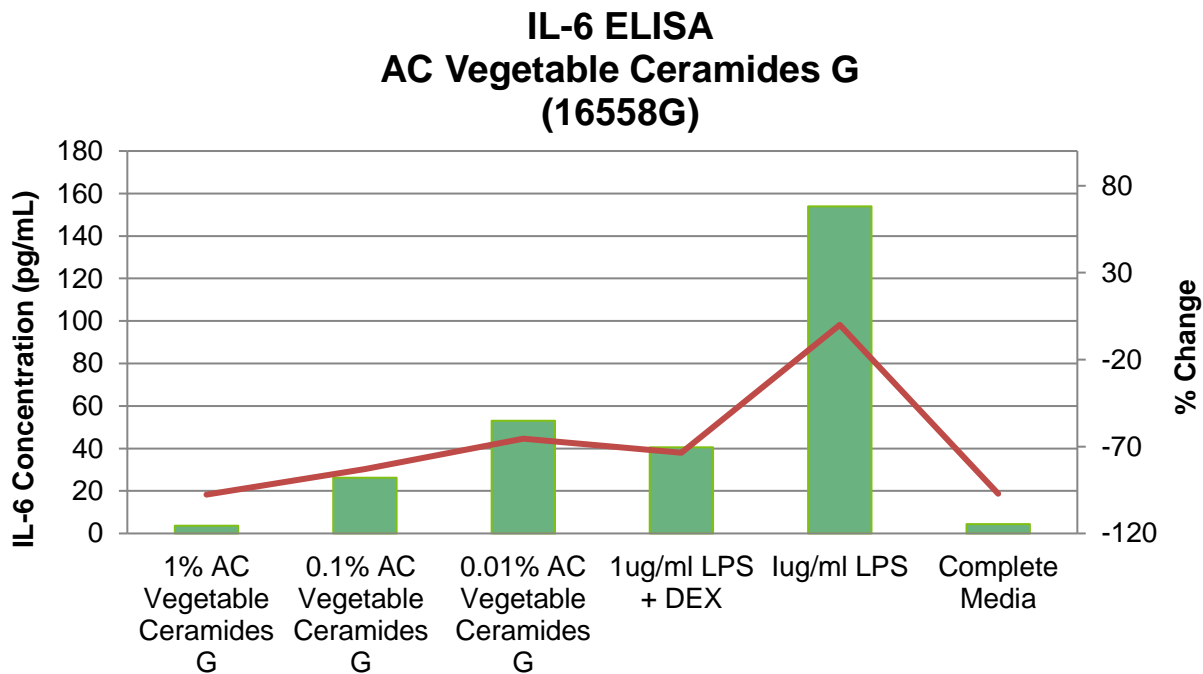
## Results

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

**AC Vegetable Ceramides G** at a concentration of 0.01% was able to decrease IL-6 production.

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

$$\text{Percent (\%) Change} = \frac{\text{IL 6 Concentration}_{\text{sample}} - \text{IL 6 Concentration}_{1\mu\text{M/mL LPS}}}{\text{IL 6 Concentration}_{1\mu\text{M/mL LPS}} \times 100$$



**Figure 1: AC Vegetable Ceramides G** -treated fibroblasts IL-6 concentrations and percent change

## Discussion

As shown in figure 1, **AC Vegetable Ceramides G (16558G)** exhibited anti-inflammatory effects on LPS-treated fibroblasts. 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. It can therefore be concluded that at normal use concentrations **AC Vegetable Ceramides G** enhances soothing and anti-aging properties.

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