



IL-6 ELISA Analysis

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Tradename: Phytofuse Rejuvenate®

Code: 16882

CAS #: 93384-40-8

Test Request Form #: 899

Lot #: NC140813-H

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- κ 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 **Phytofuse Rejuvenate®**-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.

<p>This information is presented in good faith but is not warranted as to accuracy of results. Also, freedom from patent infringement is not implied. This information is offered solely for your investigation, verification, and consideration.</p>



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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); 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 **Phytofuse Rejuvenate**[®] 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 **Phytofuse Rejuvenate**[®] 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.

Phytofuse Rejuvenate® at a concentration of 1% was able to decrease IL-6 production.

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

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

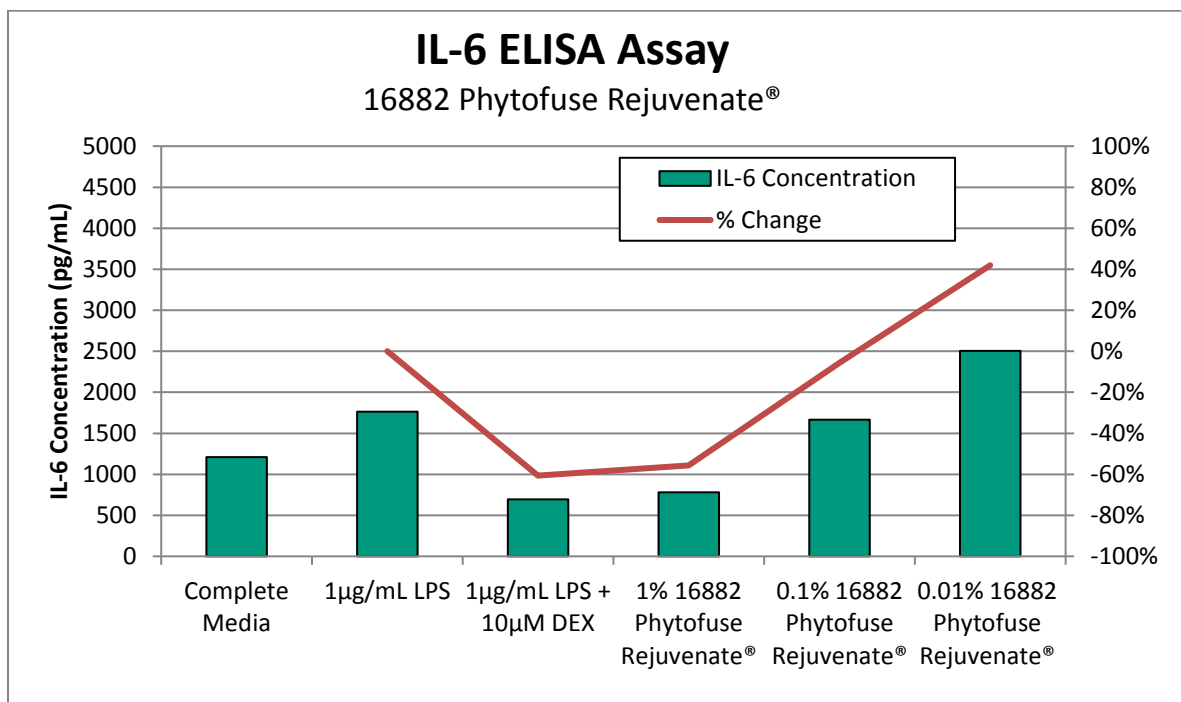


Figure 1: Phytofuse Rejuvenate®-treated fibroblasts IL-6 concentrations and percent change

Discussion

As shown in figure 1, **Phytofuse Rejuvenate®** (code 16882) 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 **Phytofuse Rejuvenate®** enhances soothing and anti-aging properties.