



## IL-6 ELISA Analysis

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**Tradename:** ACB Yogurt Dermal Respiratory Factor PF

**Code:** 20224PF

**CAS #:** 68333-15-3

**Test Request Form #:** 938

**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- $\kappa$ 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 **ACB Yogurt Dermal Respiratory Factor PF** 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

- |                                  |   |
|----------------------------------|---|
| <b>A. Kit:</b>                   | IL-6 ELISA Kit (Biosource; KAC1261)   |
| <b>B. Incubation Conditions:</b> | 37°C at 5% CO <sub>2</sub> and 95% relative humidity (RH)   |
| <b>C. Equipment:</b>             | Forma humidified incubator; ESCO biosafety laminar flow hood; Microplate Reader; Pipettes   |
| <b>D. Cell Line:</b>             | Normal Human Dermal Fibroblasts (NHDF) (Lonza; CC-2511)   |
| <b>E. Media/Buffers:</b>         | Dulbecco's Modified Eagle Medium (DMEM); Penicillin-Streptomycin (50U-50mg/mL); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS); Amphotericin (45pg/mL) |
| <b>F. Culture Plate:</b>         | Falcon flat bottom 12-well tissue culture treated plates  |
| <b>G. Reagents:</b>              | Lipopolysaccharide (LPS) (1µg/mL)   |
| <b>H. Other:</b>                 | 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 **ACB Yogurt Dermal Respiratory Factor PF** 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 as 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 **ACB Yogurt Dermal Respiratory Factor PF** treated-fibroblasts was determined by extrapolation from the standard curve and expressed in pg/mL.

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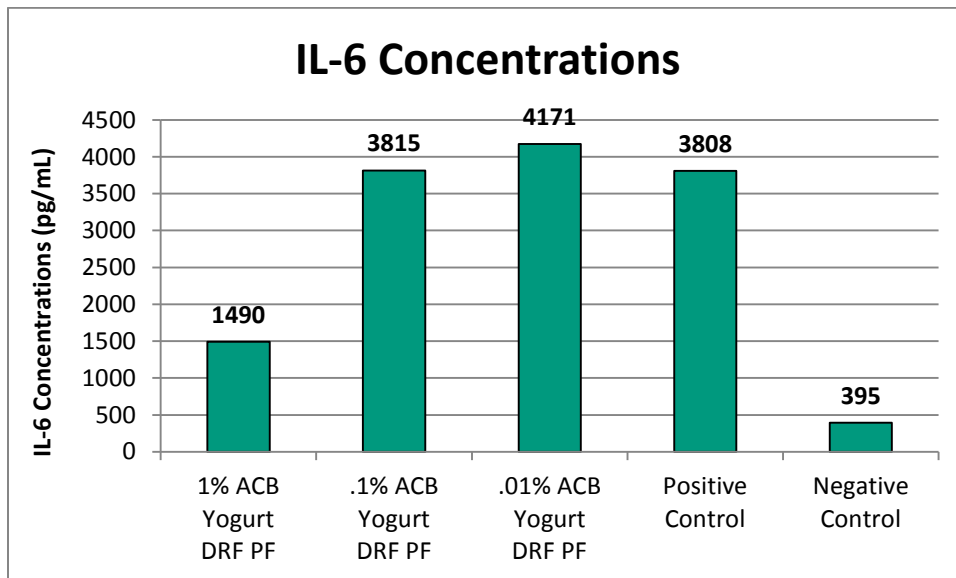
## Results

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

**ACB Yogurt Dermal Respiratory Factor PF** at a 1% concentration was able to decrease IL-6 production compared to our positive controls.

IL-6 levels are expressed by the following formula:

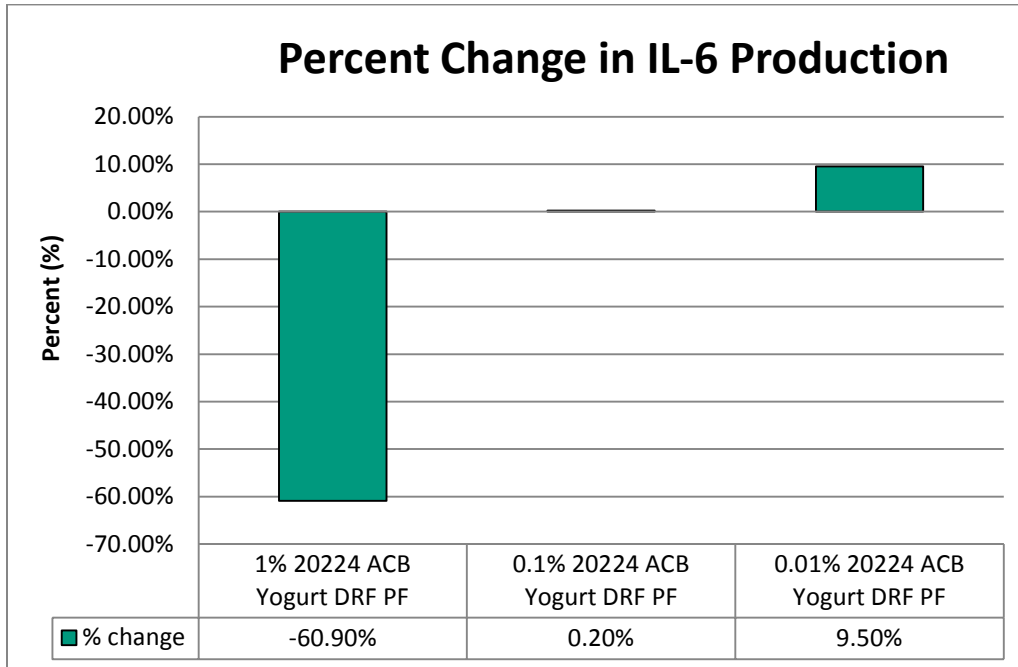
$$= \text{Average}_{IL-6 \text{ Concentrations}} \times \text{Dilution Factor}$$



**Figure 1: ACB Yogurt Dermal Respiratory Factor PF-treated fibroblasts IL-6 concentrations**

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

$$= \frac{\text{Positive Control}_{Avg.Concentration} - \text{Sample}_{Avg.Concentration}}{\text{Positive Control}_{Avg.Concentration}} \times 100$$



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

## Discussion

As shown in figure 1, **ACB Yogurt Dermal Respiratory Factor PF** exhibited anti-inflammatory effects on LPS-treated fibroblasts. As expected, the changes in IL-6 production using **ACB Yogurt Dermal Respiratory Factor PF** appear 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 **ACB Yogurt Dermal Respiratory Factor PF** is suitable for cosmetic applications designed to provide soothing and anti-aging properties.