info@activeconceptsllc.com • +1 (704)-276-7100 • Fax: +1 (704)-276-7101

IGF-1 ELISA Analysis

Tradename: AC AlfalfaBoost

Code: 20988

CAS #: 84082-36-0 & 68333-16-4 (or) 92128-79-5

Test Request Form #: 8425

Lot #: N221128G

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Test Performed:

Insulin-Like Growth Factor (IGF)-1 Enzyme-Linked Immunosorbent Assay (ELISA)

Introduction

Insulin-Like Growth Factor-1 (IGF-1) is a 70 amino acid polypeptide that plays a large role in mediating the actions of growth hormones. IGF-1 is important in prenatal development, growth into adulthood, and metabolic control. In addition, it is an important mitogen and regulator of the cell cycle and apoptosis[1]. More recently, it has been shown that IGF-I stimulates hair follicle (HF) growth, maintains the anagen stage, and postpones the catagen stage[2].
Increasing the concentration of IGF-1 is believed to stimulate the dermal papilla cells and the hair follicle, thus resulting in follicle elongation and hair growth.

Insulin-Like Growth Factor (IGF)-1 ELISA was conducted to assess the changes in IFG-1 levels in **AC AlfalfaBoost** -treated *in vitro* cultured Human Dermal Papilla Cells.

Assay Principle

This ELISA utilizes a colorimetric reaction employing antibodies with antigen specificity to human IGF-1. Monoclonal antibodies specific for IGF-1 epitopes are coated on a microtiter plate. In positive samples, IGF-1 will bind to these antibodies and are tagged a second time with another IGF-1-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 IGF-1 in each sample.

1. Quantikine® ELISA. Human IGF-I Immunoassay. *R&D Systems v05.00* (2014)
2. Won-Soo Lee, *et. al.*. Effect of IGF-I on Hair Growth Is Related to the Anti-Apoptotic Effect of IGF-I and Up-Regulation of PDGF-A and PDGF-B. *Ann Dermatol*, 24(1): 26-31 (2012)

info@activeconceptsllc.com • +1 (704)-276-7100 • Fax: +1 (704)-276-7101

Materials

A. Kit:	IGF-1 ELISA Kit (Abcam; ab108873)
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; Centrifuge
D. Cell Line:	Human Hair Follicle Dermal Papilla Cells (HFDPCs) (Cell Applications Inc; 602K-05a)
E. Media/Buffers:	Dermal Papilla Growth Media (DPGM) (Cell Applications Inc.); Collagen Coating Solution (Cell Applications Inc.)
F. Culture Plate:	Falcon flat bottom 24-well tissue culture treated plates
G. Reagents:	L-Ascorbic acid 2-phosphate, AA2P (Sigma; A8960-5G)
H. Other:	Sterile disposable pipette tips; wash bottles

Methods

Human dermal papilla cells were seeded into collagen coated 24-well tissue culture plates and allowed to grow to confluency in Dermal Papilla Growth Media (DPGM). 1%, 0.1%, 0.01% concentrations of **AC AlfalfaBoost** were added to Dermal Papilla Growth Media and incubated with HDPCs for 3 days. DPGM containing 0.25mM L-Ascorbic acid 2-phosphate was used as the positive control.

Standards were prepared in concentrations ranging from 96 ng/mL to 0pg/mL. 50µL of standards, controls, and samples were added to appropriate wells. After a two hour incubation at 2-8°C and washing, 200µL of 1X wash buffer was added to all wells and aspirated. The wash steps were repeated 4-5 times. 50µLof iX Biotinylated Insulin like Growth Factor 1 Antibody was added to each well and incubated for 2 hours. The wash step as described above was repeated. 50µL of 1X SP Conjugate was added to each well and incubated for 30 minutes. The wash step as described above was repeated. 50µL of Chromogen substrate was added to each well and incubated for 25 minutes. 50µL stop solution was added to added to each well. 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 IGF-1 concentration of **AC AlfalfaBoost** treated-HDPCs was determined by extrapolation from the standard curve and expressed in ng/mL.

info@activeconceptsllc.com • +1 (704)-276-7100 • Fax: +1 (704)-276-7101

Results

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

AC AlfalfaBoost at a concentration of 0.01% was able to increase IGF-1 production.

IGF-1 production percent increase is calculated by the following formula:

$$Percent\ (\%)\ Change = \frac{IGF1\ Concentration_{Sample} - IGF1\ Concentration_{AA2P}}{IGF1\ Concentration_{AA2P}} \times 100$$

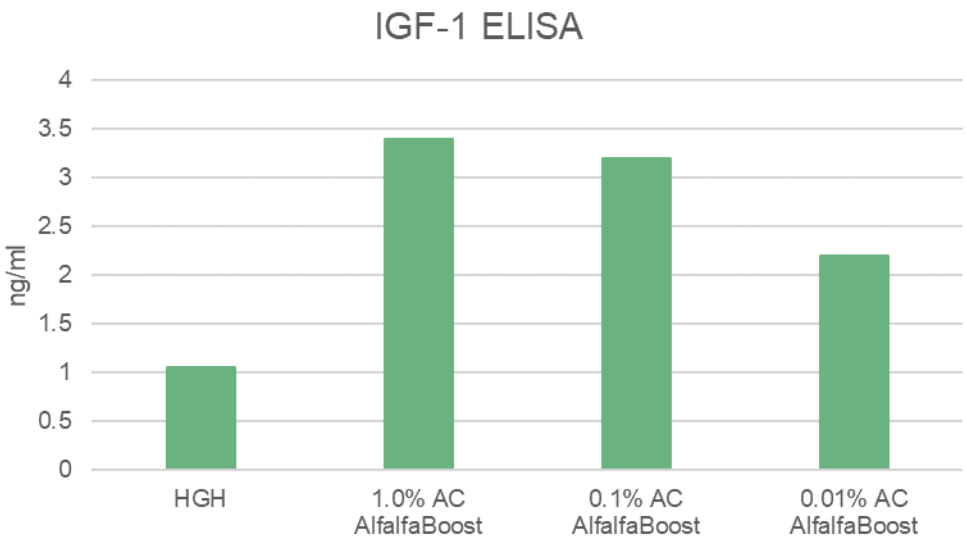

Figure 1: AC AlfalfaBoost -treated dermal papilla cells IGF-1 concentrations and percent change

Discussion

As shown in figure 1, **AC AlfalfaBoost** (20988) created an environment conducive to hair growth and follicle stimulation. This increased concentration of IGF-I available for stimulation of the dermal papilla cells promotes hair elongation and maintenance of the anagen phase. It can therefore be concluded that at normal use concentrations **AC AlfalfaBoost** can increase the growth of hair and hair follicles.