Elastin ELISA

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Tradename: AC Griffonia Lysate Advanced

Code: 16634

CAS #: 999999-99-4

Test Request Form #: 5871

Lot #: N200617K

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

Test Performed:
Elastin Enzyme-Linked Immunosorbent Assay (ELISA)

Introduction

Elastic like fibers, such as Elastin, comprise part of the extracellular matrix and confer elasticity to organs and tissues including the heart, skin, lungs, ligaments, and blood vessels. The encoded protein is rich in hydrophobic amino acids such as glycine and proline, which form mobile hydrophobic regions bounded by crosslinks between lysine residues. Degradation products of the encoded protein, known as elastin-derived peptides or elastokines, bind the Elastin receptor complex resulting in the migration and proliferation of dermal fibroblasts. Deletions and mutations in this gene present as wrinkled or loose skin along with easy bruising and scaring. Increasing production of Elastin is believed to slow down degradation of the skin matrix and, possibly, stimulate its replenishment.

Accordingly, an Elastin ELISA was conducted to assess the *in vitro* effect of **AC Griffonia Lysate Advanced** on the extracellular release of Elastin from human dermal fibroblasts.

Assay Principle

The Elastin ELISA Kit operates by mixing an affinity tag labeled capture antibody with a reporter conjugated detector antibody that binds to Elastin. After Elastin is labeled, an immobilized complex is formed upon binding to anti-tag antibodies coating the wells. Unbound materials are removed during washing steps, and adding 3,3',5,5'-tetramethylbenzidine (TMB) Development Solution generates a blue color that is catalyzed by horseradish peroxidase (HRP). Adding Stop Solution to samples finalizes the color change from blue to yellow and absorbance is measured. The signal generated is proportional to the amount of bound Elastin and concentrations are calculated. Cell culture supernatants (media) from all conditions are collected to determine secreted levels of Elastin.

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Materials

A. Kit:	Human Elastin ELISA Kit (Abcam; ab239433)*
B. Incubation Conditions:	37°C, 5% CO_2, and 95% relative humidity (RH)
C. Equipment:	Forma humidified incubator, ESCO biosafety laminar flow hood, Synergy HT Microplate reader; Pipettes; Light microscope
D. Cell Line:	Normal Human Adult Dermal Primary Fibroblasts (ATCC; PCS-201-012)*
E. Media/Buffers:	Fibroblast Basal Medium (PCS-201-030)*; Fibroblast Growth Kit (PCS-201-041)*; Antibody Diluent CPI; 10X Wash Buffer PT; Sample Diluent NS; Sample Diluent 50BS
F. Reagents:	Dexamethasone (DEX) (10 µM); Retinol (RET) (10 µM); Elastin Recombinant Protein; 10X Elastin Capture Antibody; 10X Elastin Detector Antibody; TMB Development Solution; Stop Solution
G. Culture Plate:	Flat Bottom 12-Well Tissue Culture Treated Plate; Pre-Coated 96 Well Microplate (12 x 8 well strips)
H. Software:	Excel Analysis ToolPak (Microsoft)
I. Other:	Sterile disposable pipette tips; 15 mL Conical tubes; 1.7 mL Microcentrifuge tubes

*Or suitable alternatives, subject to change without notice based off vendor availability

Methods

Human dermal fibroblasts were seeded into a 6-well tissue culture plate and allowed to grow to confluency in complete media (CM). 0.01% and 0.1% concentrations of **AC Griffonia Lysate Advanced** in CM were added to cells and placed at 37°C. CM was used as the untreated control, while DEX (10 µM) and RET (10 µM) were added to CM and utilized as positive controls. After 48 hours, media was collected according to the manufacturer's instructions and utilized in the Human Elastin ELISA Kit (ab239433).

Elastin standards were prepared ranging in concentrations from 0 pg/mL to 12,000 pg/mL. After adding 50 µL of standards and samples to the appropriate wells, 50 µL of the Antibody Cocktail was added to all wells. Following a one-hour incubation at room temperature, all wells were washed three times with 350 µL of 1x Wash Buffer PT and 100 µL of TMB Development Solution was added to each well. After a 10-minute incubation in the dark, 100 µL of Stop Solution was added to each well. The optical density was read at 450 nm on a Synergy HT Microplate Reader.

Assays were repeated three separate times with each sample run in duplicate. Duplicates for each replicate were averaged, and the average of all three experiments is displayed. Data was analyzed using a one-way ANOVA with statistical significance accepted at $p \leq 0.05$. A standard curve was created by reducing the data and generating a linear curve fit. The secreted Elastin concentrations of **AC Griffonia Lysate Advanced**-treated fibroblasts were determined by extrapolation from the standard curve and expressed in pg/mL. Percent changes in Elastin are relative to CM and were calculated with the following formula:

$$Percent\ Change = \frac{Elastin\ Concentration_{Sample} - Elastin\ Concentration_{CM}}{Elastin\ Concentration_{CM}} \times 100$$

Results

The data obtained from this study met criteria for a valid assay and the positive controls performed as anticipated. Compared to untreated fibroblasts, DEX and RET increased secreted Elastin concentrations. Fibroblasts treated with **AC Griffonia Lysate Advanced** at 0.01% and 0.1% enhanced the levels of secreted Elastin above the values of CM alone.

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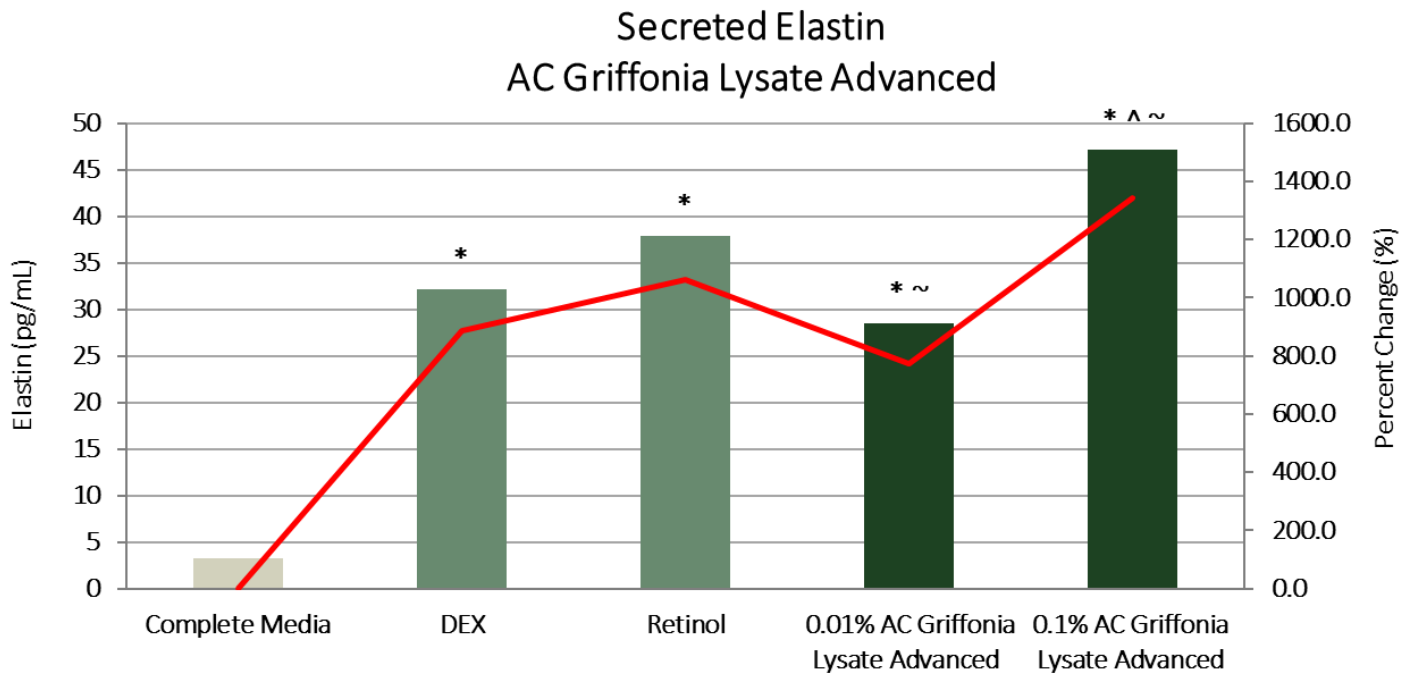

Figure 1. Concentrations of Elastin secreted from **AC Griffonia Lysate Advanced**-treated fibroblasts. * indicates significance ($p \leq 0.05$) compared to untreated fibroblasts. ^ indicates significance ($p \leq 0.05$) compared to DEX.

Table 1. Results from one-way ANOVA Statistical Analysis of Elastin Secreted Compared to untreated fibroblasts. * indicates significance ($p \leq 0.05$) compared to untreated fibroblasts. ^ indicates significance ($p \leq 0.05$) compared to DEX.

	Complete Media	0.01% **AC Griffonia Lysate Advanced**	0.1% **AC Griffonia Lysate Advanced**
Complete Media	-----	0.021*	0.037*
DEX	0.029*	> 0.05	0.010^
Retinol	0.028*	0.016~	0.007~

Discussion

As shown in Figure 1, fibroblasts exposed to DEX and RET demonstrated 886% and 1062% increases in Elastin secretion compared to untreated fibroblasts, respectively. This data demonstrates the levels of Elastin released from fibroblasts can be augmented with compounds known to increase Elastin synthesis. Similarly, treatment with **AC Griffonia Lysate Advanced** at 0.01% and 0.1% demonstrated 774% and 1344% increases in the levels of Elastin released fibroblasts, respectively. These data demonstrate **AC Griffonia Lysate Advanced** has the ability to augment fibroblast Elastin secretion.

These increases in Elastin synthesis indicate stimulation, migration, and proliferation of skin fibroblasts. This provides an 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 Griffonia Lysate Advanced** enhances skin matrix replenishment and anti-aging properties as well as slowing skin matrix degradation.