Human Elastin ELISA Analysis

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

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*
Principle Investigator: *Jennifer Goodman*

Test Performed:
Human Elastin (ELN) 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 and other receptors and stimulate migration and proliferation of monocytes and skin fibroblasts. Elastokines can also contribute to cancer progression. Deletions and mutations in this gene are associated with supravalvular aortic stenosis (SVAS) and autosomal dominant cutis laxa, which presents itself in the form of 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.

A Human Elastin (Elastin) ELISA was conducted to assess the changes in Elastin levels in **AC Griffonia Lysate Advanced** treated *in vitro* cultured human dermal fibroblasts.

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

Assay Principle

This ELISA utilizes a colorimetric reaction employing antibodies with antigen specificity to Elastin. Monoclonal antibodies specific for Elastin are coated on a microtiter plate. In positive samples, Elastin will bind to these antibodies and are tagged a second time with another Elastin-specific (Adivin) 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 enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm.The standard curve provides a reference from the OD readings for the amount of Elastin in each sample.

Materials

A. Kit:	Human Elastin ELISA Kit (BioMatik EKU03852)
B. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity (RH)
C. Equipment:	HERAcell Vios 160i Incubator (Thermo Scientific); ESCO Biosafety Laminar Flow Hood; Synergy H1 Microplate Reader (BioTek Instruments, Winooski, VT); Gen5 software (BioTek Instuments, Winooski, VT); Pipettes
D. Cell Line:	Normal Human Neo-Natal Dermal Primary Fibroblasts (HDFn) (ATCC PCS-201-010)
E. Media/Buffers:	Fibroblast Basal Medium (ATCC PCS-201-030); Fibroblasts Growth Kit- Low Serum (ATCC PCS-201-041) Penicillin-Streptomycin (50U-50mg/mL); Standard Diluent BioMatik); 1X Wash Buffer (BioMatik)
F. Culture Plate:	Falcon flat bottom 6-well tissue culture treated plates
G. Reagents:	All Trans Retinol (10uM) (MP Bio 190268); Dexamethasone-acetate (DEX) (10µM) (MP Bio 190040); Detection Reagents A & B (BioMatik); Elastin Standard (BioMatik)
H. Other:	Sterile disposable pipette tips; wash bottles

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

Methods

Human dermal fibroblasts were seeded into 6-well tissue culture plates and allowed to grow to confluency in complete media. 0.1% and 0.01% concentrations of **AC Griffonia Lysate Advanced** were added to complete media containing and incubated with fibroblasts for 48 hours. Complete media was used as the untreated control, while DEX (10µM) and Retinol (10µM) were used as the positive controls.

Elastin Standards were prepared in concentrations ranging from 100pg/mL to 0pg/mL. 100µL of standards, controls, and samples were added to appropriate wells. The plate was sealed and incubated at 37°C for one hour. After a one hour incubation at 37°C, the liquid of each well was removed and 100 µL of the working solution of Detection Reagent A was added to each well. The plated was sealed and incubated for one hour at 37°C. After the second hour of incubation, solution was aspirated and the entire plate was washed with 350 µL of 1X wash solution allowing the wash solution to sit for 1-2 minutes before being removed. This step was repeated a total of three times. After the last wash, the plate was inverted and blotted against absorbent paper. 100µL of working solution of Detection Reagent B was added to each well and the plate was sealed and incubated at 37°C for 30 minutes. Post incubation, the aspiration and wash process was repeated five more times. 90 µL of the Substrate Solution was added to each well and the plated was sealed and incubated at 37°C protected from light for a maximum of 20 minutes while monitoring color development. 50 µL of stop solution was added to stop the reaction after 10-20 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 Elastin Concentration of **AC Griffonia Lysate Advanced** treated-fibroblasts was determined by extrapolation from the standard curve and expressed in pg/mL.

Results

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

AC Griffonia Lysate Advanced at a concentration of 0.01% was able to increase Elastin production.

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

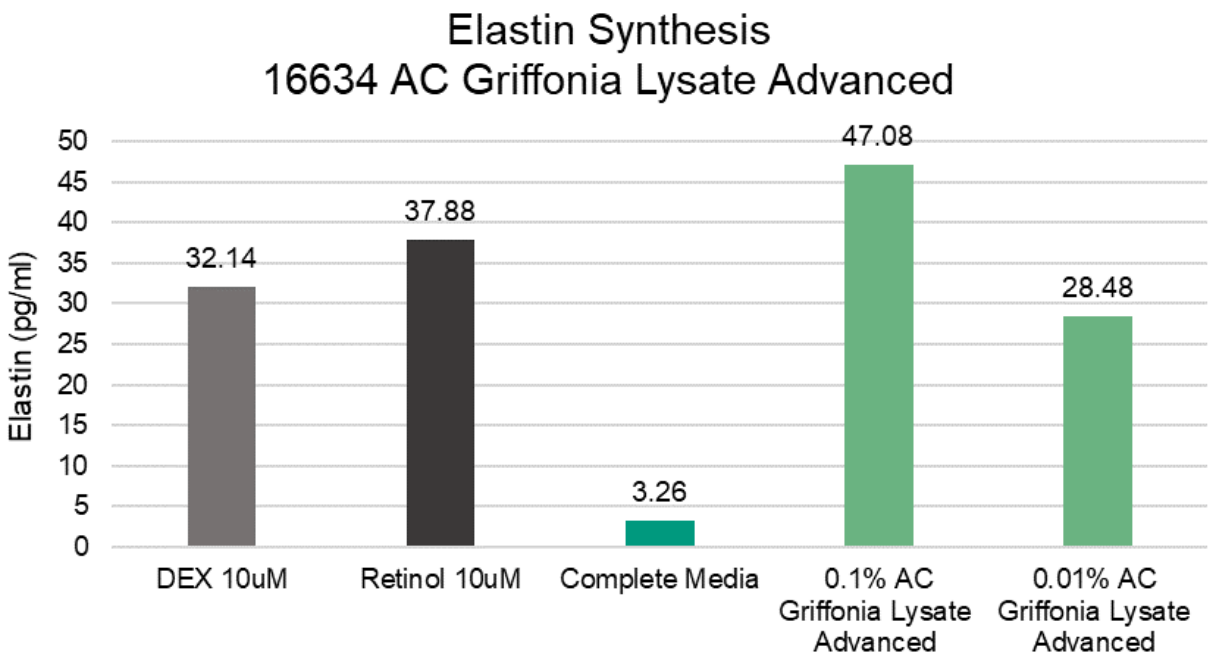

Figure 1: AC Griffonia Lysate Advanced -treated neonatal fibroblasts Elastin concentrations

Discussion

As shown in figure 1, **AC Griffonia Lysate Advanced** exhibited increased Elastin synthesis in neonatal fibroblasts.

This increase in Elastin synthesis indicates 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.