Elastin ELISA

ACTIVE CONCEPTS LLC
107 Technology Drive, Lincolnton | NC 28092 USA
• info@activeconceptsllc.com • Phone: +1-704-276-7100 • Fax: +1-704-276-7101 • https://activeconceptsllc.com/

Tradename: AC PomeaShield

Code: 16935

CAS #: 7732-18-5 & 84961-57-9 & 1686112-36-6 (or) 68333-16-4

Test Request Form #: 9262

Lot #: N220316M

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Drumwright*
Principle Investigator: *Daniel Shill*

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 PomeaShield** on the intracellular synthesis 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. Solubilized fibroblast lysates from all conditions are collected to determine intracellular 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. 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 12-well tissue culture plate and allowed to grow to confluency in complete media (CM). 0.01%, 0.1% and 1.0% concentrations of **AC PomeaShield** 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, fibroblast lysates were collected according to the manufacturer's instructions and utilized in the Human Elastin ELISA Kit (ab239433). All conditions were measured in duplicate.

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.

A standard curve was created by reducing the data and generating a linear curve fit. The intracellular Elastin concentrations of **AC PomeaShield**-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 intracellular Elastin concentrations. Fibroblasts treated with **AC PomeaShield** at 0.01%, 0.1%, and 1.0% enhanced the levels of intracellular Elastin above the values of CM alone.

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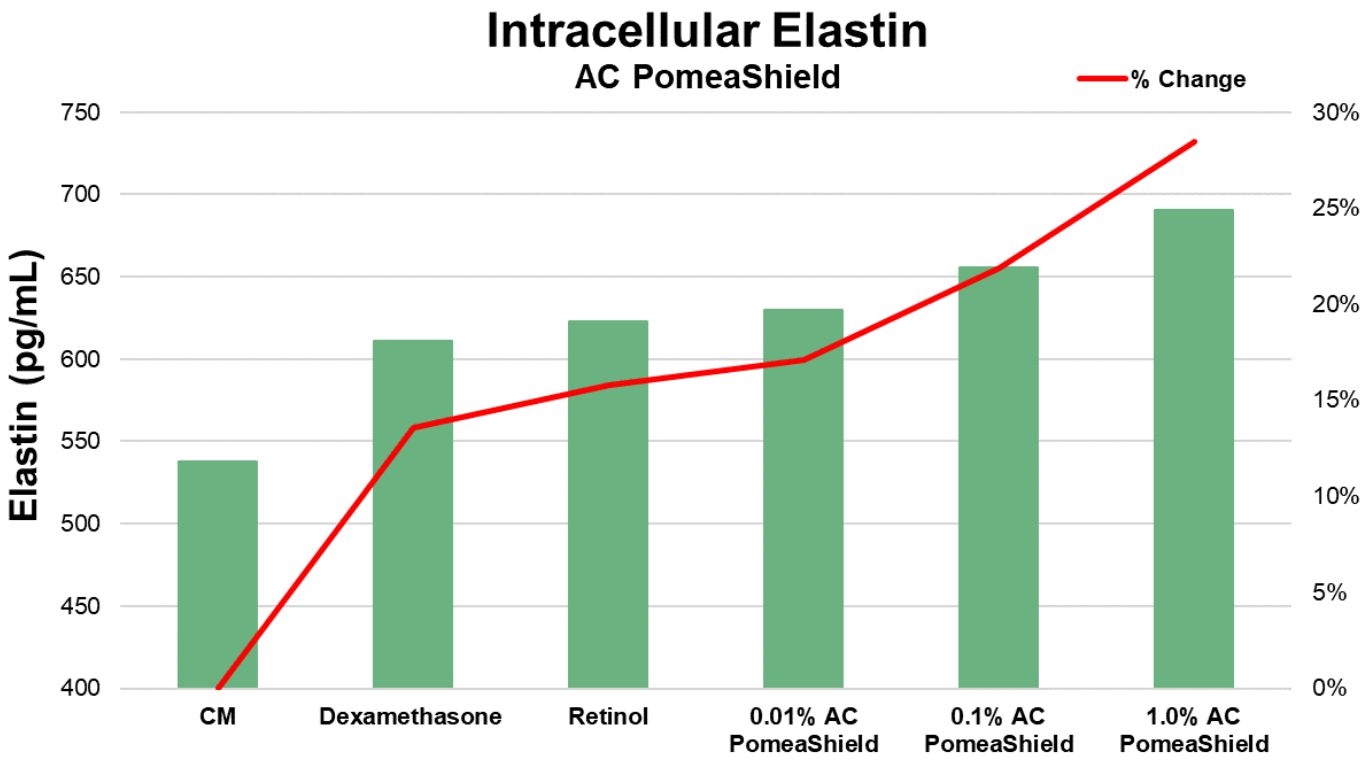

Figure 1. Intracellular concentrations of Elastin from **AC PomeaShield** -treated fibroblasts.

Discussion

As shown in Figure 1, DEX and RET exposure increased intracellular Elastin concentrations by 14% and 16% compared to untreated fibroblasts, respectively. This data demonstrates intracellular Elastin levels in fibroblasts can be enhanced with compounds known to stimulate Elastin deposition. Likewise, treatment with **AC PomeaShield** at 0.01%, 0.1%, and 1.0% augmented intracellular Elastin concentrations by 17%, 22%, and 28% compared to fibroblasts, respectively. These data demonstrate **AC PomeaShield** has the ability to increase Elastin levels in fibroblasts.

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 PomeaShield** enhances skin matrix replenishment and anti-aging properties as well as slowing skin matrix degradation.