CYP1A1 Assay

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 ExoEternol

Code: 60200

CAS #: 7732-18-5 & 68333-16-4 (or) 92128-79-5 & 90082-61-4 (or) 68132-21-8 & 123465-35-0 (or) 8002-43-5 & 68333-16-4 (or) 1686112-36-6 (or) 9015-54-7

Test Request Form #: 13043

Lot #: N250701O

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Daniel Shill*
Principal Investigator: *Hannah Stade*

Test Performed:
CYP1A1 Assay

Introduction

CYP1A1 is a cytochrome gene responsible for metabolizing foreign compounds and endogenous substances. Under environmental stress, CYP1A1 activates cellular detoxification pathways to protect against pollutants, but increases reactive oxygen species (ROS) and inflammation production. Alternatively, suppression of CYP1A1 under normal conditions promotes cellular homeostasis and differentiation while limiting excessive oxidative stress and inflammation. As a result, CYP1A1 activity is context-dependent with potential to be either protective or damaging to the skin.

Retinoids such as retinol and retinoic acid are popular skin ingredients because they suppress CYP1A1 activity resulting in improved skin repair, barrier function, and anti-aging benefits. While these ingredients provide great skin benefits, they are not ideal for sensitive skin as they can be irritating if used frequently or at too high of concentrations. Therefore, a cosmetic ingredient designed to provide the same benefits while reducing the undesirable side effects is critical.

Accordingly, a CYP1A1 Assay was conducted to assess the *in vitro* effect of **AC ExoEternol** on CYP1A1 activity in human dermal keratinocytes.

Assay Principle

The CYP1A1 Assay utilizes a cell-permeable luminogenic substrate to elucidate CYP1A1 activity. Intracellular CYP1A1 enzymes convert the substrate to luciferin which is released by the cells and detected with a luciferin detection reagent. The light output of the luciferase reaction is proportional to the CYP1A1 activity.

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/

Materials

A. Kit:	P450-Glo™ CYP1A1 Assay System (Promega; V8751)
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 Epidermal Keratinocytes (ATCC; PCS-200-011)*
E. Media/Buffers:	Keratinocyte Basal Medium (ATCC; PCS-200-030)*; Keratinocyte Growth Kit (ATCC; PCS-200-040)*
F. Culture Plate:	Flat Bottom 96-Well Tissue Culture Treated Plate; 96 Well Opaque White Microplate
G. Software:	Excel Analysis ToolPak (Microsoft)
H. Other:	Sterile disposable pipette tips; 2 mL Microcentrifuge tubes

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

Methods

Human epidermal keratinocytes were seeded into a 96-well tissue culture plate and allowed to grow to confluency in complete media (CM). 0.01% and 0.05% concentrations of **AC ExoEternol** in CM were added to cells and placed at 37°C. CM was used as the untreated control. Wells void of cells were used as a background control. After 48 hours, media was discarded, and cells were washed once with PBS. Fresh CM containing diluted luminogenic CYP substrate was added to all wells including the background wells. Following a three-hour incubation at 37°C, 25 µL of media from each well was transferred to a clean 96-well microplate. Next, 25 µL of luciferin detection reagent was added to each well and incubated at room temperature for 20 minutes. Luminescence was read 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 $p \leq 0.05$. Net signals were calculated by subtracting averaged background luminescence values from each test well. CYP1A1 activity is expressed as relative luminescence units (RLU) and displayed as percent change relative to CM and calculated with the following formula:

$$Percent\ Change = \frac{CYP1A1\ Acitivty_{Sample} - CYP1A1\ Acitivty_{CM}}{CYP1A1\ Acitivty_{CM}} \times 100$$

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/

Results

The data obtained met criteria for a valid assay. Compared to untreated keratinocytes, **AC ExoEternol** at 0.01% and 0.05% suppressed CYP1A1 activity.

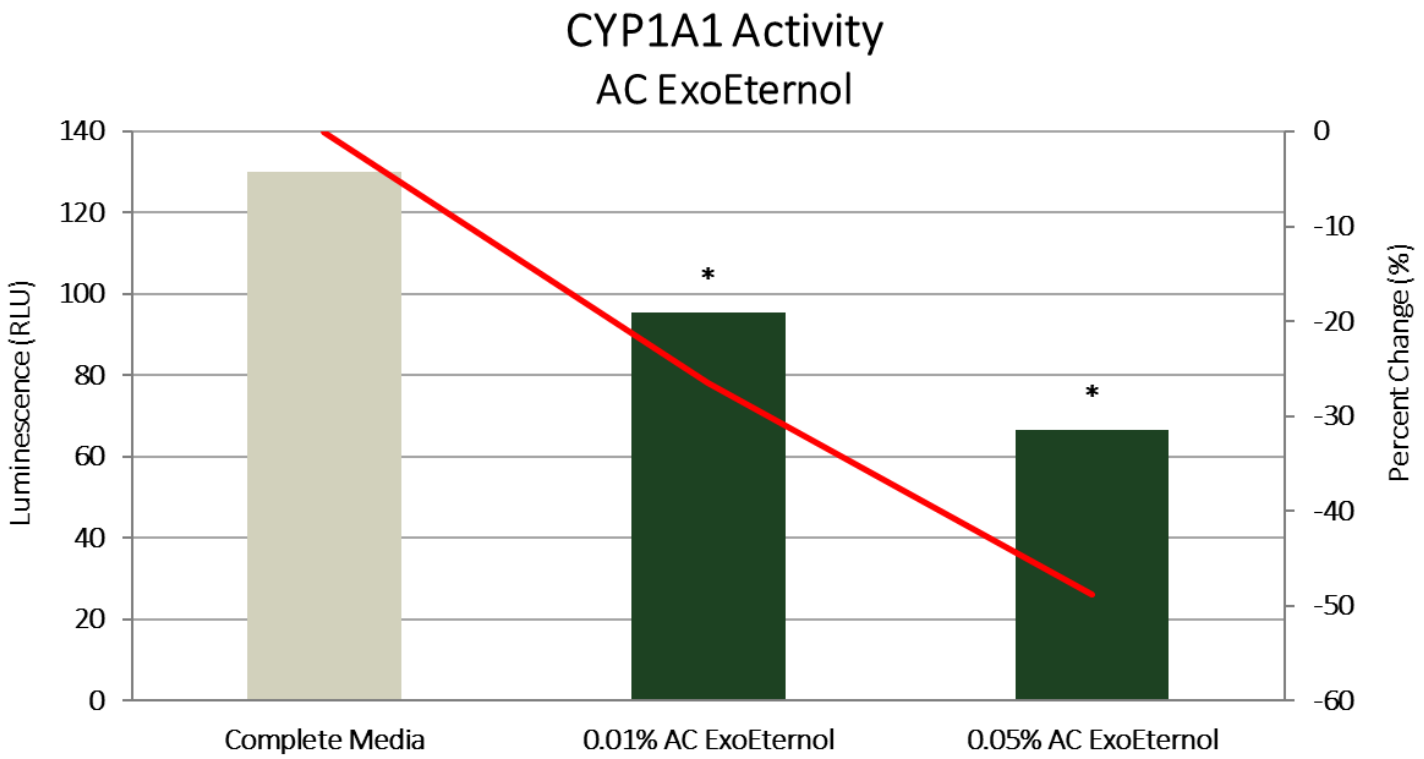

Figure 1. CYP1A1 Activity in Epidermal Keratinocytes. * indicates significance ($p \leq 0.05$) compared to untreated keratinocytes.

Table 1. Results from one-way ANOVA Statistical Analysis of CYP1A1 Activity between conditions compared. * indicates significance ($p \leq 0.05$) compared to untreated keratinocytes.

	Complete Media	0.01% **AC ExoEternol**	0.05% **AC ExoEternol**
Complete Media	-----	0.033*	0.013*

Discussion

As shown in Figure 1, keratinocytes exhibit CYP1A1 activity under normal culture conditions. Comparatively, treatment with **AC ExoEternol** at 0.01% and 0.05% demonstrated 27% and 49% reductions in keratinocyte CYP1A1 activity, respectively (Table 1). These data demonstrate **AC ExoEternol** blunts CYP1A1 expression in keratinocytes.

Taken together, these data indicate **AC ExoEternol** promotes barrier function, skin repair, and provides anti-aging effects through reduced CYP1A1 activity. Reductions in CYP1A1 activity under normal conditions indicate cellular homeostasis in keratinocytes with reduced ROS and inflammatory stress. Collectively, **AC ExoEternol** enhances skin homeostasis and provides anti-aging properties by suppressing CYP1A1 activity.