Glycolysis 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 ExoVitalize

Code: 60193

CAS #: 7732-18-5 & 8016-20-4 & 90244-99-8 & 123465-35-0 (or) 8002-43-5

Test Request Form #: 10232

Lot #: N230627A

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

Test Performed:
Glycolysis (L-Lactate) Assay

Introduction

Cellular metabolism can occur with (aerobic) or without (anaerobic) oxygen to generate ATP (adenosine triphosphate), the molecule that is the end byproduct of metabolism. The stored energy within ATP is harvested and utilized by cells to maintain homeostasis as it is required for cellular migration and proliferation, molecular transportation across membranes, and the biosynthesis of cellular components. Aerobic metabolism generates a large amount of ATP slowly and can be sustained for a long period which is ideal for cellular function. On the other hand, glycolysis, or anaerobic metabolism, can produce a lot of ATP in a relatively short amount of time but cannot be sustained over long periods. The quick generation of ATP from anaerobic metabolism is advantageous when cells are stressed and need to meet the high energy demand of responding to external stimuli. Additionally, glycolysis supplies the large quantities of ATP necessary to synthesize and deposit extracellular matrix proteins. Lactate is a byproduct of glycolysis and the amount produced by cells is directly proportional to the rate of glycolysis. Accordingly, the amount of lactate released from cells can serve as a surrogate measurement of glycolysis.

An L-Lactate Assay was conducted to assess the *in vitro* effect of **AC ExoVitalize** to stimulate glycolysis in dermal fibroblasts. Activating this biological process maintains cellular homeostasis, vitality, and can be critical during cellular stress. The key active ingredients in **AC ExoVitalize**, *Citrus paradisi* (Grapefruit) Fruit Extract and *Citrullus lantus* (Watermelon) Fruit Extract, were tested to demonstrate the superior nature of bioauthentic exosomes as a delivery system.

Assay Principle

Lactate reacts with an enzyme mix, supplied in Abcam's L-Lactate Assay Kit, to generate a byproduct that interacts with a fluorescent probe that produces color. This reaction provides a specific and quantitative method for determining the amount of lactate in a given sample. 3PO (3-(3-pyridinyl)-1-(4-pyridinyl)-2E-propen-1-one), a potent glycolytic inhibitor, reduces the amount of lactate produced and is utilized as a negative control. Since lactate is the end product of glycolysis, the amount of lactate released from cells can serve as a surrogate measurement of glycolysis.

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Materials

A. Kit:	L-Lactate Assay Kit (Abcam; ab65330)*
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 Neonatal Human Dermal Fibroblasts (ATCC; PCS-201-010)*
E. Media/Buffers:	Fibroblast Basal Medium (PCS-201-030)*; Fibroblast Growth Kit (PCS-201-041)*
F. Reagents:	Assay Buffer (ab65330)*; OxiRed Probe/Lactate Probe (ab65330)*; Lactate Enzyme Mix (ab65330)*; Lactate Standard (ab65330)*; 3PO (3-(3-pyridinyl)-1-(4-pyridinyl)-2E-propen-1-one) (Cayman Chemical; 19276)*; Dimethyl Sulfoxide (DMSO)
G. Culture Plate:	96 Well Black Side/Clear Bottom Tissue Culture Treated Microplates
H. Software:	Excel Analysis Toolpak (Microsoft)
H. Other:	Sterile disposable pipette tips

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

Methods

Human neonatal dermal fibroblasts were seeded into a 96-well tissue culture microplate and grew to 80%-90% confluency in complete media (CM). 0.01%, 0.1% and 1.0% concentrations of **AC ExoVitalize** were added to CM, while 3PO was initially reconstituted in DMSO and further diluted with CM. Additionally, a solution of 0.01% *Citrus paradisi* (Grapefruit) Fruit Extract and 0.01% *Citrullus lantus* (Watermelon) Fruit Extract was prepared in CM (0.02% Fruit Extract Blend). These concentrations of *Citrus paradisi* (Grapefruit) Fruit Extract and *Citrullus lantus* (Watermelon) Fruit Extract are equivalent to the amount present in 0.1% **AC ExoVitalize**. As experimental controls, CM alone was regarded as a baseline value, and 3PO at 10 µM was utilized as a negative control to inhibit glycolysis. All conditions, tested in duplicate, were added to fibroblasts and incubated at 37°C. Following an 18-hour incubation, the media in all wells was collected and stored at -80°C. The media samples were thawed and utilized in the L-Lactate Assay Kit (ab65330) according to the manufacturer's instructions. Briefly, 50 µL of standards, controls, and samples were added to all wells. After 50 µL of the reaction mix was added to each well, the entire plate was incubated in the dark at room temperature for 30 minutes and fluorescence measurements were taken at excitation / emission wavelengths of 535 nm / 587 nm.

Three separate experiments were performed with conditions in duplicate and average values were recorded. Data was analyzed using a one-way ANOVA with statistical significance accepted at $p \leq 0.05$. Lactate values from the standards were plotted and a regression equation was determined from the standard curve. The lactate concentration of **AC ExoVitalize**-treated fibroblasts was extrapolated from the standard curve and expressed in µM. Percent change is expressed relative to CM and calculated by the following equation:

$$Percent\ Change\ (\%) = \frac{Lactate_{Sample} - Lactate_{CM}}{Lactate_{CM}} \times 100$$

Results

The data obtained from this study met criteria for a valid assay and the controls performed as anticipated. Compared to untreated fibroblasts, 3PO (10 µM) reduced lactate concentrations. Fibroblasts treated with the 0.02% Fruit Extract Blend increased lactate production by 2%, whereas all concentrations of **AC ExoVitalize** demonstrated increased lactate production in a dose dependent fashion compared to untreated fibroblasts.

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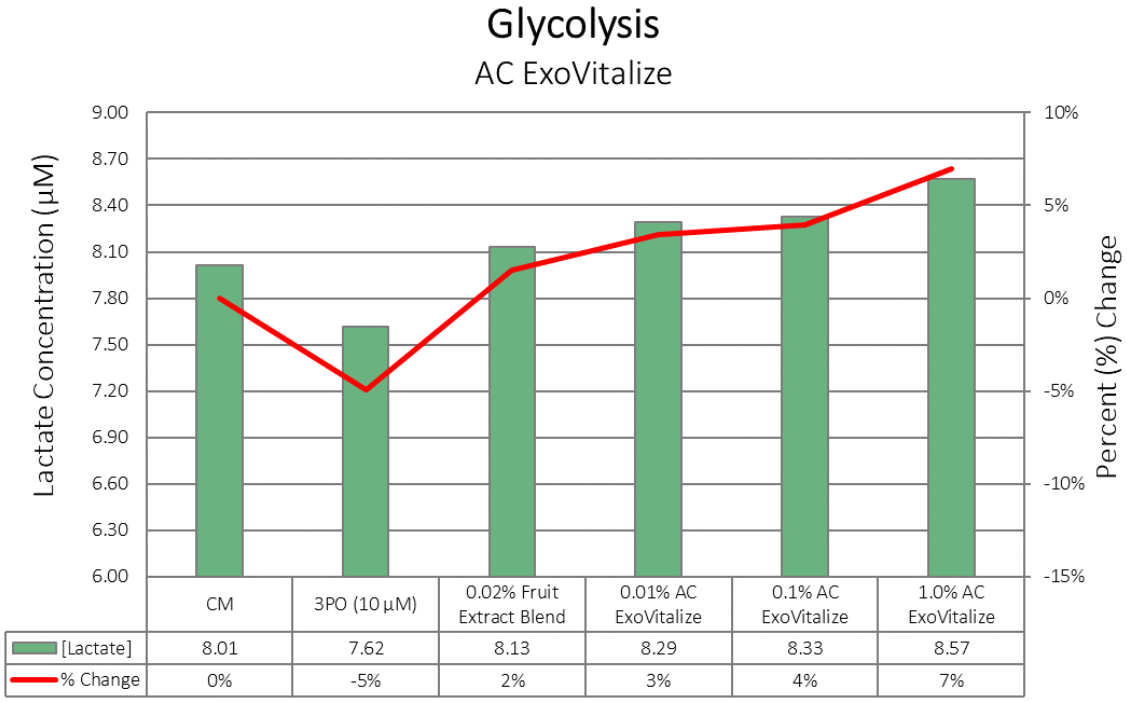

Figure 1. The effect of **AC ExoVitalize** on fibroblast lactate production.

Table 1: Results from one-way ANOVA statistical analysis. Results represent p-values between the two conditions compared.

	3PO	0.02% Fruit Extract Blend	0.01% **AC ExoVitalize**	0.1% **AC ExoVitalize**	1.0% **AC ExoVitalize**
Complete Media	< 0.001	> 0.05	0.001	0.001	< 0.001
0.02% Fruit Extract Blend	0.026	-----	0.034	0.028	0.012

Discussion

As shown in Figure 1, fibroblasts incubated with 3PO, a known glycolytic inhibitor, exhibited a 5% reduction in lactate concentrations compared to untreated fibroblasts. These data demonstrate glycolytic metabolism in fibroblasts is dynamic and can be manipulated with exogenous compounds.

Conversely, fibroblasts treated with **AC ExoVitalize** at 0.01%, 0.1%, and 1.0% demonstrated 3%, 4%, and 7% increases in lactate concentrations compared to untreated fibroblasts, respectively (Table 1). The 0.02% Fruit Extract Blend only increased lactate concentrations by 2%, highlighting the superior nature of bioauthentic exosomes as a delivery system. These data demonstrate **AC ExoVitalize** activates glycolysis in fibroblasts.

Collectively, glycolysis is a biological process that assists in critical cellular functions as ATP generation is required for cellular migration and proliferation, molecular transportation across membranes, and the biosynthesis of cellular components. Increasing glycolysis can assist in the maintenance of cellular homeostasis, vitality, and can be critical during cellular stress as large quantities of ATP are necessary for the formation of extracellular matrix proteins. In summary, these data indicate **AC ExoVitalize** stimulates glycolysis, which accelerates the biosynthesis of cellular components and can assist in attenuating the physical signs of dermal aging by reducing the formation of wrinkles through increases extracellular matrix synthesis and deposition.