

Tradename: AC CytoPure PF

Code: 20757PF

CAS #: 92128-82-0

Test Request Form #: 2269

Lot #: NC130424-B

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Test Performed:

Glutathione Assay

Introduction

Glutathione is a sulfur-rich peptide that is often present in cellular systems as reduced Glutathione (GSH). All organisms metabolize sulfur by reduction or oxidation. GSH plays a large role in many biological processes involving the maintenance of the oxidant state of protein sulfhydryl groups, detoxification of xenobiotics, and removal of hydroperoxides. Intracellular glutathione combats inflammation and slows the effects of aging via DNA, protein, and lipid synthesis as well as enzyme and amino acid transport regulation. However, concentrations of GSH decrease within the epidermis over time due to a shift in the redox reaction rate, favoring oxidation. The oxidation of sulfhydryl groups and the presence of xenobiotics and hydroperoxides in the cellular system cause a toxic environment in the cell which can lead to aging, decrease in cellular function, tumor growth, and even apoptosis. Without GSH, other antioxidants cannot function adequately to protect the body, thus GSH is a key indicator of overall cell health and toxic challenge resistance.

Accordingly, a Glutathione Assay was conducted to assess the *in vitro* effects of **AC CytoPure PF** on GSH concentrations in dermal fibroblasts.

Assay Principle

This Glutathione Assay utilizes a colorimetric reaction with a reaction rate that is proportional to the concentration of glutathione up to 2 μ M. The samples are deprotonated with 5% 5-Sulfosalicylic Acid Solution and centrifuged to remove proteins leaving GSH to be involved in the reaction. 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) is reduced continuously into 5-thio-2-nitrobenzoic acid (TNB) by GSH. TNB forms a yellow product and is photometrically measured at 412 nm. The Glutathione Disulfide (GSSG) is recycled by Glutathione Reductase and Nicotinamide Adenine Dinucleotide Phosphate (NADPH) allowing the continuous cycle. The standard curve provides a reference from the optical density (OD) readings for the amount of GSH in each sample. With an increase in GSH in the biological sample, there is an increase in TNB and therefore an increase in yellow product can be observed.

Materials

- A. Kit:** Glutathione Assay Kit (Sigma, CS0260)
- B. Incubations Conditions:** 37°C at 5% CO₂ and 95% relative humidity (RH)
- C. Equipment:** Forma humidified incubator; ESCO biosafety laminar flow hood; Synergy H1 Microplate reader (BioTek Instruments, Winooski, VT); Gen5 software (BioTek Instruments, Winooski, VT); Pipettes
- D. Cell Line:** Normal Human Dermal Fibroblasts (ATCC; PCS-201-012)*
- E. Media/Buffers:** Fibroblast Basal Medium (PCS-201-030)*; Fibroblast Growth Kit (PCS-201-041)*; Phosphate Buffered Saline (PBS); Glutathione Assay Buffer 5X; NADPH (40mg/ml); Dimethyl Sulfoxide (DMSO) (1.5mg/ml)
- F. Culture Plate:** Flat bottom 96-well tissue culture treated plates
- G. Reagents:** Glutathione Reductase; Glutathione Standard Stock Solution (GSH) (10mM); 5,5'-Dithiobis(2-nitrobenzoic acid) [DTNB]; 5-Sulfosalicylic Acid (SSA) (5%)
- H. Other:** Sterile disposable pipette tips
- I. Software:** Excel Analysis ToolPack (Microsoft)
- *Or suitable alternatives, subject to change without notice based off vendor availability

Methods

Human dermal fibroblasts were seeded into 96-well tissue culture plates and allowed to grow to confluency in complete media. 1.0% concentrations of **AC CytoPure PF** were added to complete media and incubated with fibroblasts for 72 hours. L-cysteine (1 mg/mL) is known to have similar properties at GSH and was utilized as a positive control. Following incubation, cells were washed with PBS and then suspended in PBS. Cells were spun down, supernatant removed, pellet volume measured, and 3 volumes of 5% SSA solution were added to the pellet and vortexed. The pellet suspensions were subjected to two freeze-thaw cycles, followed by a centrifuge spin at 10,000 x g for 10 minutes. The supernatants were collected and utilized according to kit directions.

Briefly, standards were prepared in concentrations ranging from 0.0312 nmoles to 0.5 nmoles. The reagent blank of 5% SSA Solution was used as the negative control. 10 µL of standards, controls, and samples were added to appropriate wells. 150 µL of the Working Mixture was added to each well and mixed. The plate was incubated for 5 minutes at room temperature (25°C) before 50 µL of diluted NADH Solution was added to each well and mixed. The optical density was read at 412 nm at 1 minute intervals for a total of 5 minutes on the Synergy H1 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 GSH concentration of **AC CytoPure PF** treated-fibroblasts was determined by extrapolation from the standard curve and expressed in nmoles/mL.

Results

The data obtained met criteria for a valid assay and the controls performed as anticipated. Compared to untreated fibroblasts, **AC CytoPure PF** at 1.0% demonstrated increased GSH concentrations.

Glutathione Concentration AC CytoPure PF

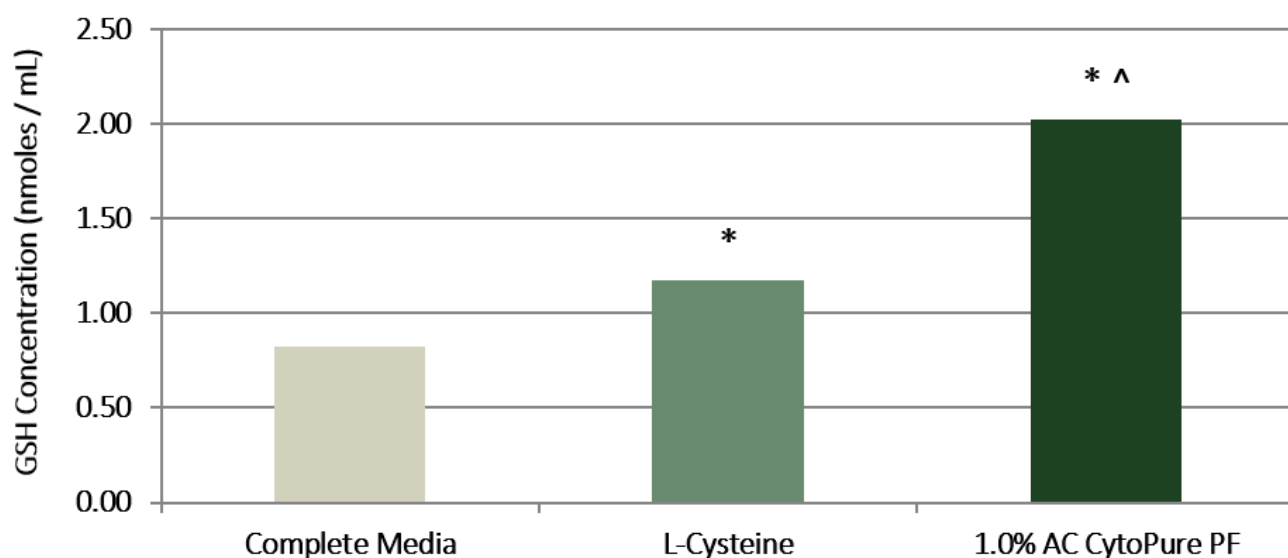


Figure 1. Fibroblast Glutathione Levels. * indicates significance ($p \leq 0.05$) compared to Complete Media. ^ indicates significance ($p \leq 0.05$) compared to L-Cysteine.

Table 1. Results from one-way ANOVA Statistical Analysis Compared to Complete Media. * indicates significance ($p \leq 0.05$) compared to Complete Media. ^ indicates significance ($p \leq 0.05$) compared to L-Cysteine.

	L-Cysteine	1.0% AC CytoPure PF
Complete Media	0.033*	0.002*
L-Cysteine	-----	0.025^

Discussion

As shown in Figure 1, 1.0% **AC CytoPure PF** exhibited 146% increases in GSH concentrations in human dermal fibroblasts. This increase in GSH indicates an increase in antioxidant capacity that is known to reduce toxins and oxide species in the intracellular environment. Working to void toxins from the cell and increase overall cellular health can have a positive effect on cellular viability and attenuating the signs and symptoms of aging by regulating DNA synthesis.

Collectively, **AC CytoPure PF** is capable of promoting anti-aging via GSH enhancement that in turn increases overall cell health and function.