



Intracellular Calcium Assay

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Tradename: ABS Pap-Ango Enzyme PF

Code: 10268PF

CAS #: 84012-30-6 & 90063-86-8

Test Request Form #: 8488

Lot #: 8349600

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*

Study Director: *Maureen Danaher*

Principle Investigator: *Daniel Shill*

Test Performed:

Intracellular Calcium Assay

Introduction

Intracellular calcium concentrations, often linked to light exposure, play a key role in skin health and immune response as it can help reduce inflammation, with a direct effect on tissue repair and keratinocyte function. Calcium also helps maintain skin moisture levels and promotes the production of antioxidants.

Keratinocytes, making up 90-95% of the epidermis, are a physical and chemical barrier for the skin by minimizing UV radiation damage, water loss, and microbial, viral, and parasitic invasion. Accordingly, keratinocytes must progressively proliferate, differentiate, and migrate to maintain skin barrier integrity and homeostasis. Provided keratinocyte proliferation, differentiation, and migration are calcium dependent, intracellular calcium levels can serve as a surrogate for skin health.

A Fluo-4 Direct™ Calcium Assay was performed to assess changes in intracellular calcium levels in **ABS Pap-Ango Enzyme PF**-treated human epidermal keratinocytes *in vitro*.

Assay Principle

The Fluo-4 Direct™ Calcium Assay Kit detects intracellular calcium by utilizing Fluo-4, a cell-permeant fluorescent dye. Upon entering the cell and binding to free calcium, Fluo-4 exhibits fluorescence signals when excited at 488nm providing an indication of intracellular calcium. A suppression dye, which reduces non-intracellular fluorescence and inhibits extracellular transport, is included to eliminate background fluorescence from the complete media and prevent the movement of Fluo-4 out of the cell. An increase in fluorescent signal intensity generated by labeled calcium molecules is indicative of higher levels of intracellular calcium.

Materials

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|----------------------------------|---|
| A. Kit: | Fluo-4 Direct™ Calcium Assay Kit (ThermoFisher; F10471) |
| B. Incubation Conditions: | 37°C at 5% CO ₂ and 95% Relative Humidity (RH) |
| C. Equipment: | Forma humidified incubator; ESCO biosafety laminar flow hood; Light microscope; Synergy H1 Microplate Reader; Pipettes |
| 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: | Falcon Flat Bottom 96-Well Tissue Culture Treated Plates |
| G. Reagents: | Calcium chloride (CaCl ₂) (Carolina; 85-1800); Ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA) (EMD Millipore; 32-462-6) |
| H. Other: | Sterile disposable pipette tips |

Methods

Testing began September 20, 2021 and concluded September 24, 2021. Human epidermal keratinocytes were seeded into a 96-well tissue culture plate and allowed to grow to confluency in complete media (CM). 0.01%, 0.1%, and 1.0% concentrations of **ABS Pap-Ango Enzyme PF** were added to complete media and incubated with keratinocytes. As experimental controls, complete media containing 1mM CaCl₂ was utilized as a positive control to increase intracellular calcium levels, and 2mM EGTA in the presence of 1mM CaCl₂ was utilized as a negative control to reduce the CaCl₂-induced increases in intracellular calcium. After a 60-minute incubation at 37°C, 50µL of 2X Fluo-4 Direct™ calcium reagent loading solution was added to all wells and the plate was returned to 37°C for 60 minutes followed by 30 minutes at room temperature. Next, fluorescence measurements (excitation 494nm, emission 516nm) were taken on a Synergy H1 Microplate Reader. Intracellular Calcium results are shown as Relative Fluorescence Units (RFU) and expressed as percentage change, calculated by the following equation:

$$\text{Percent (\%)Change} = \frac{RFU_{Control} - RFU_{Sample}}{RFU_{Control}} \times 100$$

Results

The data obtained from this study met criteria for a valid assay and the experimental controls performed as anticipated. Keratinocytes treated with **ABS Pap-Ango Enzyme PF** at 0.01%, 0.1%, and 1.0% augmented intracellular calcium levels compared to complete media alone.

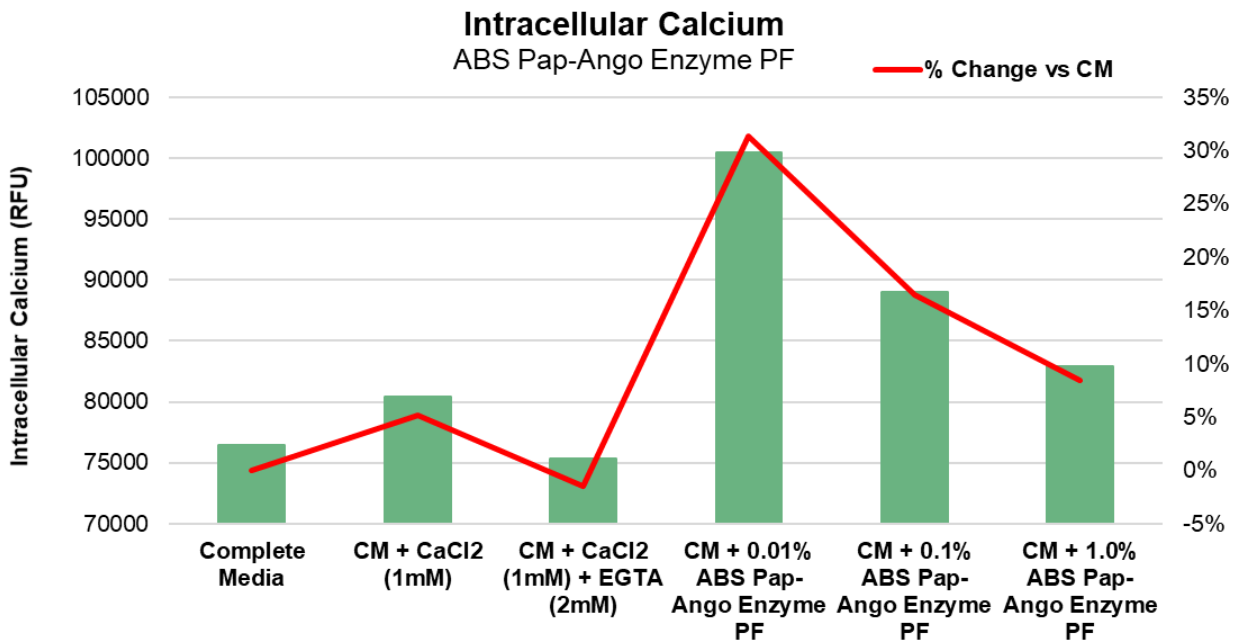


Figure 1: Relative Keratinocyte Intracellular Calcium Levels.

Discussion

As shown in Figure 1, adding CaCl₂ to complete media increased keratinocyte intracellular calcium by 5% compared to complete media alone. However, when EGTA was introduced to CaCl₂ and complete media, intracellular calcium was reduced by 6% compared to CaCl₂ and complete media, establishing our experimental model. **ABS Pap-Ango Enzyme PF** at 0.01%, 0.1%, and 1.0% enhanced keratinocyte intracellular calcium levels by 31%, 16%, and 8% compared to complete media alone, respectively.

Collectively, these data demonstrate that **ABS Pap-Ango Enzyme PF** increases intracellular calcium, an indicator of keratinocyte proliferation, differentiation, and migration, which contributes to the maintenance of skin barrier integrity and homeostasis.