

**Tradename:** ACB Modified Pomegranate Enzyme PF

**Code:** 20440PF

**CAS #:** 84961-57-9 & 1686112-10-6 (or) 84775-94-0 (or) 9015-54-7

**Test Request Form #:** 9564

**Lot #:** 8755200

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

**Study Director:** *Maureen Drumwright*

**Principal Investigator:** *Jennifer Goodman*

**Test Performed:**

Cellular Renewal Study: Wash-Off

**Introduction**

As the largest human organ, the skin's integrity is critical to properly function as a physical barrier and maintenance of a healthy appearance for aesthetics. The epidermis constantly undergoes major self-renewal as the superlayer of cells are lost by desquamation and replaced by cells in the basal layers. The constant replacement of cells mitigates the negative long-term effects of ultraviolet light damage and harmful agents (chemicals, pollutants, etc.) on the skin. Aiding in the processes of cellular renewal can improve the skin's physical appearance as well as function as a protective barrier.

Accordingly, a cellular renewal study was conducted to evaluate the ability of **ACB Modified Pomegranate Enzyme PF** to accelerate skin cell replacement by assessing changes in pigmentation.

**Study Principle**

Dermal Dye Max™ (active ingredient: dihydroxyacetone (DHA) / glycerone) is applied to the skin and creates artificially high pigmented skin. The controls and test materials are applied to the artificially pigmented areas and pigmentation is measured overtime. The artificially pigmented areas provide a model to assess cellular renewal by measuring pigmentation with decreases in pigmentation representing cellular renewal.

**Materials**

- A. Equipment:** DermaLab Skin Combo (Skin Color Probe); Digital Camera
- B. Products:** Dermal Dye Max™ (Alpine Valley Naturals); Base Cleanser (Cetaphil® Daily Facial Cleanser All Skin Types); Glycolic Acid (positive control)
- C. Software:** Excel Analysis ToolPak (Microsoft)

## Methods

Ten volunteers between the ages of 28 and 40, who were known to be free of any skin pathologies with Fitzpatrick skin types I to III, participated in this study (Table 1).

**Table 1.** The Fitzpatrick Classification of Skin Types Chart<sup>1</sup>

Fitzpatrick Skin Type Descriptions*	
Skin Type	Description
I	Always burns, never tans
II	Burns easily, tans minimally
III	Burns moderately, tans to light brown
IV	Burns minimally, tans to moderate brown
V	Rarely burns, tans to dark
VI	Never burns, least sensitive to changes

\*Adapted from The Surgeon General's Call to Action to Prevent Skin Cancer

Five test sites were identified on the volar forearm of participants. The first test site was identified as the Comparative Skin Site, and no dye nor treatment were applied to this site to demonstrate normal pigmentation readings. Dermal Dye Max™ was applied to the remaining four test sites and allowed to develop for 24 hours prior to baseline readings. After dye development and prior to initial treatment application, baseline DermaLab pigmentation index readings were taken for all five identified sites. The skin test site conditions and treatments are described below (Table 2). The Untreated Dye Control received Dermal Dye Max™ but no treatment to demonstrate normal cellular renewal.

All cleanser formulations and the Base Lotion were adjusted to a direct pH of 4.3-4.8 (Table 2). The Base Cleanser utilized in this study was Cetaphil® Gentle Cleanser for All Skin Types. Approximately 0.2 g of each treatment was applied to three 2 cm x 2 cm respective locations on the volar forearm. After each treatment was applied, each test site was thoroughly rinsed with warm water and patted dry with a paper towel. Pigmentation readings and images were taken every 24 hours until the active test site returned to baseline. After each daily reading, treatment of each respective test site was performed following the same parameters listed above

**Table 2.** Descriptions of the Conditions and Treatments for each Skin Test Site

Skin Test Site	Condition	Dermal Dye Max™ Application?	Treatment / Test Material Application Description	Lotion pH
1	Comparative Skin Site	No	None	N/A
2	Untreated Dye Control	Yes	None	N/A
3	Base Cleanser	Yes	Base Cleanser	4.4
4	Glycolic Acid	Yes	5.0% Glycolic Acid in Base Cleanser	4.7
5	ACB Modified Pomegranate Enzyme PF	Yes	5.0% ACB Modified Pomegranate Enzyme PF in Base Cleanser	4.6

The percent change of Pigmentation values was calculated for each test site at every timepoint relative to Baseline values, using the following equation:

$$\text{Percent Change (\%)} = \frac{\text{Pigmentation Index}_{\text{Day}} - \text{Pigmentation Index}_{\text{Baseline}}}{\text{Pigmentation Index}_{\text{Baseline}}} \times 100$$

Cumulative Cellular Renewal, represented by the Pigmentation Index values and relative to the Untreated Dye Control, was calculated as area under the curve (AUC) by using the following equation:

$$AUC = \frac{1}{2} \sum_{i=0}^{n-1} (t_{i+1} - t_i) (\text{Pigmentation Index}_i + \text{Pigmentation Index}_{i+1})$$

For pigmentation measurements  $\text{Pigmentation Index}_1$  and  $\text{Pigmentation Index}_2$  at times  $t_1$  and  $t_2$ , the AUC between those two time points is equivalent to the product of difference in time and the average of the two Pigmentation measurements. Provided Pigmentation Index values decreased over time AUC was calculated as an inverse and presented as a positive value to demonstrate the amount of cellular relative to the Untreated Dye Control.

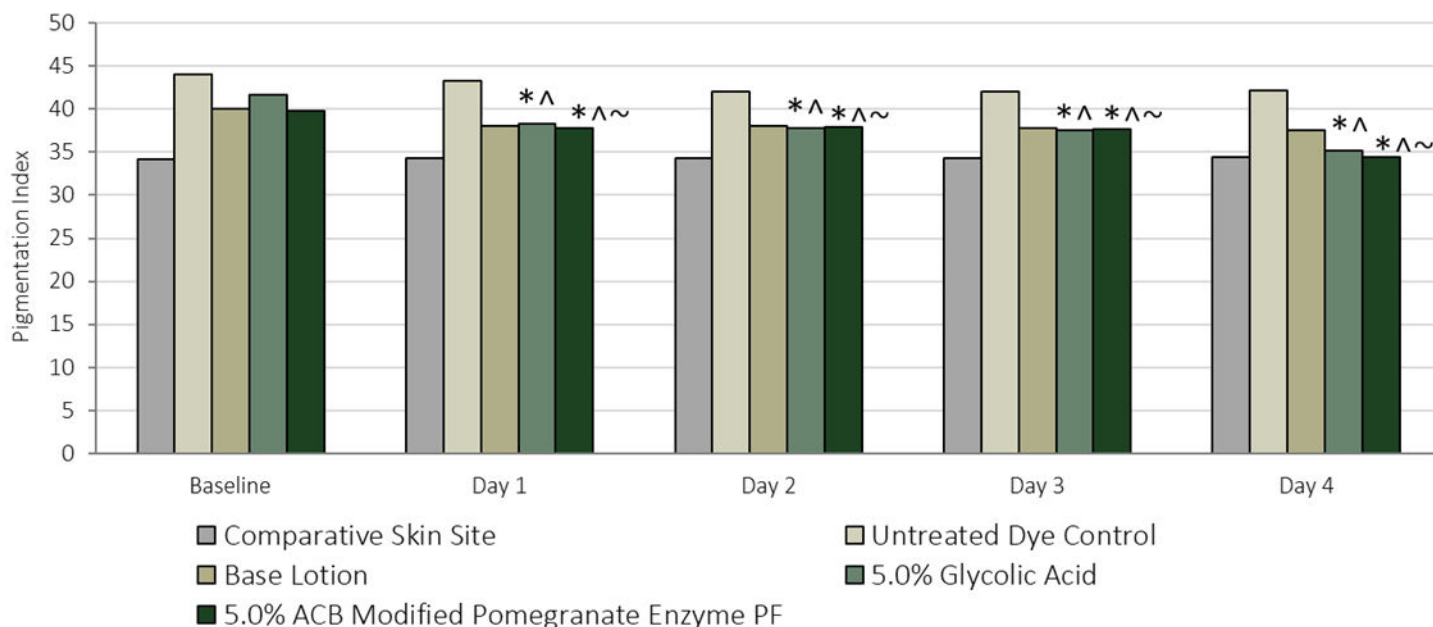
Percent change in Cumulative Cellular Renewal with respect to the Base Lotion was calculated for each test site, using the following equation:

$$\text{Percent Change (\%)} = \frac{\text{Condition}_{AUC} - \text{Base Lotion}_{AUC}}{\text{Base Lotion}_{AUC}} \times 100$$

## Results

The data obtained from this study met criteria for a valid study as the Comparative Skin Site, Untreated Dye Control, and Glycolic Acid performed as anticipated. Application of 5.0% **ACB Modified Pomegranate Enzyme PF** accelerated cellular renewal as pigmentation values returned to the baseline levels of the Comparative Skin Site after four days.

## Cellular Renewal ACB Modified Pomegranate Enzyme PF



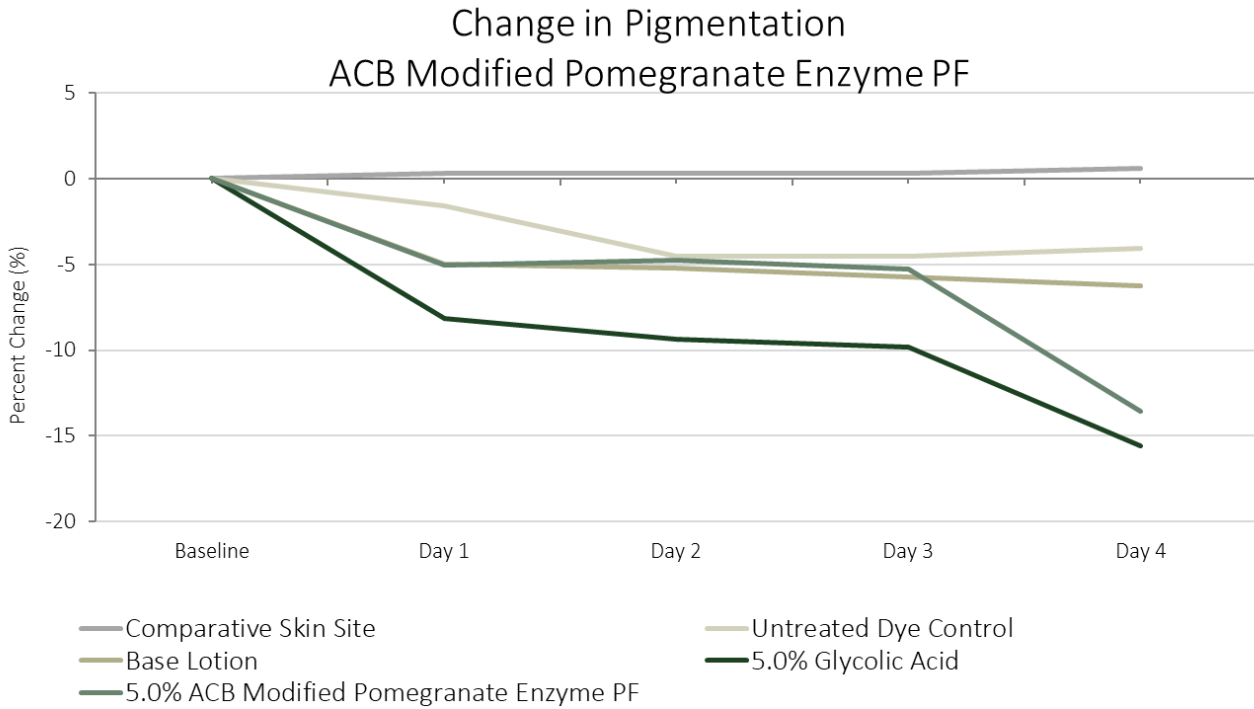
**Figure 1.** Cellular Renewal of Pigmentation Index Values Overtime. \* indicates significance ( $p \leq 0.05$ ) compared to Baseline within the same condition. ^ indicates significance ( $p \leq 0.05$ ) compared to Untreated Dye Control. ~ indicates significance ( $p \leq 0.05$ ) compared to Base Lotion.

**Table 3.** Results from T-test Analyses of Pigmentation Index Values from Baseline to Day Four. \* indicates significance ( $p \leq 0.05$ ) compared to Baseline.

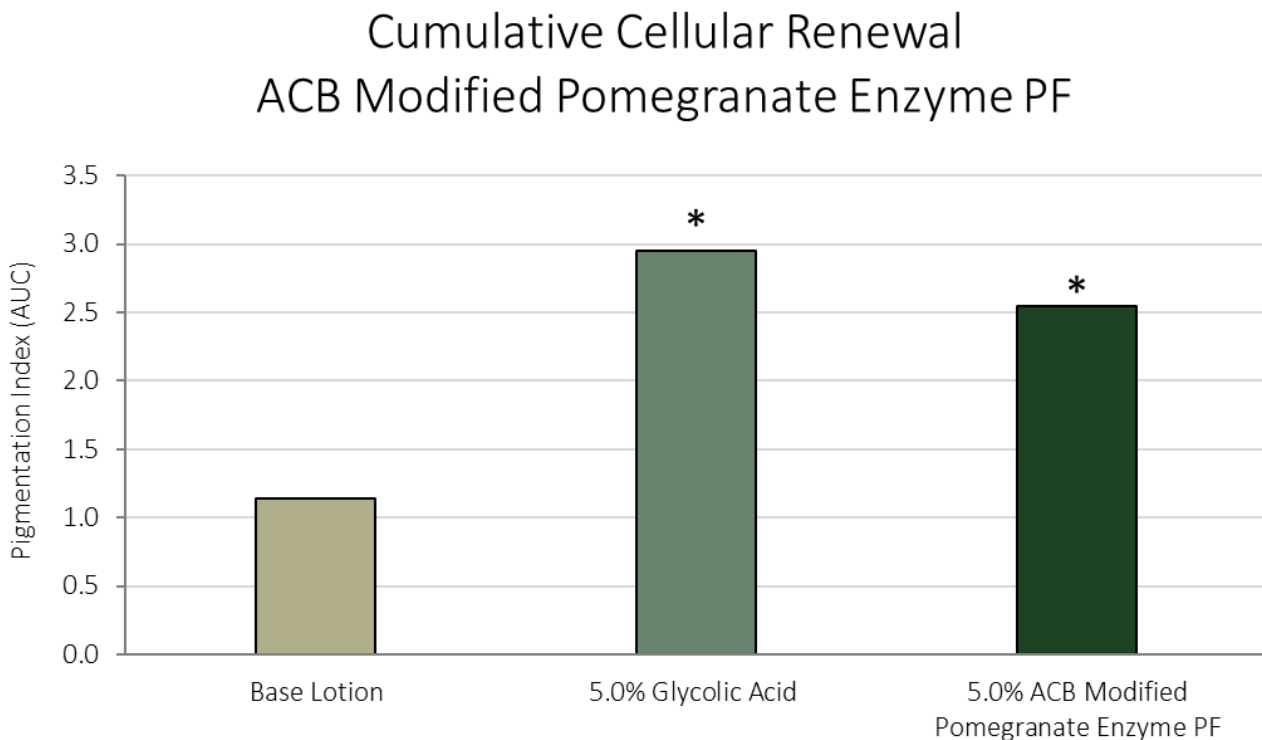
	Comparative Skin Site	Untreated Dye Control	Base Lotion	5.0% Glycolic Acid	5.0% ACB Modified Pomegranate Enzyme PF
<b>P-value</b>	0.070	0.444	0.051	0.0102*	0.031*

**Table 4.** Results from T-test Analyses of Pigmentation Index Values After Four Days of Application. ^ indicates significance ( $p \leq 0.05$ ) between conditions.

	Untreated Dye Control vs Base Lotion	Untreated Dye Control vs 5.0% Glycolic Acid	Untreated Dye Control vs 5.0% ACB Modified Pomegranate Enzyme PF	Base Lotion vs 5.0% Glycolic Acid	Base Lotion vs 5.0% ACB Modified Pomegranate Enzyme PF	5.0% Glycolic Acid vs 5.0% ACB Modified Pomegranate Enzyme PF
<b>P-value</b>	0.094	0.026^	0.024^	0.068	0.042~	0.234



**Figure 2.** Percent Change in Pigmentation Relative to Baseline Readings. \* indicates significance ( $p \leq 0.05$ ) compared to Baseline.



**Figure 3.** Cumulative Cellular Renewal in Pigmentation Relative to the Untreated Dye Control. ^ indicates significance ( $p \leq 0.05$ ) between conditions. Percent change in cumulative cellular renewal is relative to Base Lotion.

**Table 5.** Results from One-way ANOVA Analysis of Cumulative Cellular Renewal. \* indicates significance ( $p \leq 0.05$ ) between conditions.

	Base Lotion vs 5.0% Glycolic Acid	Base Lotion vs 5.0% <b>ACB Modified Pomegranate Enzyme PF</b>	5.0% Glycolic Acid vs 5.0% <b>ACB Modified Pomegranate Enzyme PF</b>
P-value	0.035*	0.001*	0.196

## Discussion

The ability of **ACB Modified Pomegranate Enzyme PF** to accelerate skin cellular renewal was assessed through changes in pigmentation. As shown in Figure 1 and 2, the Dermal Dye Max™ artificially augmented pigmentation values at baseline measurements. After four days, the Untreated Dye Control demonstrated a 4% reduction in pigmentation indicating normal cellular renewal (Figures 1, 2). Similarly, the Base Lotion exhibited a 6% decrease in pigmentation demonstrating a slight increase in normal cellular renewal. After four days of application, 5.0% Glycolic Acid induced a 16% decrease in pigmentation as expected. However, 5.0% **ACB Modified Pomegranate Enzyme PF** elicited a 14% reduction in pigmentation and outperformed the positive control.

Additionally, 5.0% **ACB Modified Pomegranate Enzyme PF** induced a significant change in pigmentation after four days compared to the Untreated Control and Base Lotion (Figures 1, 2; Table 4). Similarly, there were no differences in pigmentation between 5.0% Glycolic Acid and 5.0% **ACB Modified Pomegranate Enzyme PF** after four days of application (Figures 1, 2; Table 4). These results indicate 5.0% **ACB Modified Pomegranate Enzyme PF** accelerates cellular renewal at a similar rate compared to 5.0% Glycolic Acid.

Similar results are shown when the data is displayed to illustrate the collective effect of each treatment when the Untreated Dye Control pigmentation values are accounted for. As shown in Figure 3, 5.0% **ACB Modified Pomegranate Enzyme PF** produced the largest change in cumulative cellular renewal compared to the Base Lotion and 5.0% Glycolic Acid. Although there were no significant differences between 5.0% Glycolic Acid and 5.0% **ACB Modified Pomegranate Enzyme PF** in terms of Cumulative Cellular Renewal, 5.0% **ACB Modified Pomegranate Enzyme PF** produced the largest change compared to the Base Lotion and 5.0% Comparative Material 2 (Figure 3; Table 5).

Taken together, these results indicate **ACB Modified Pomegranate Enzyme PF** enhances cellular renewal when added to personal care applications at recommended use levels. Collectively, **ACB Modified Pomegranate Enzyme PF** accelerates the processes of cellular renewal which can improve the skin's physical appearance with a healthier and more vibrant skin as well as function as a protective barrier to help reverse the signs of aging.

## References

1. Sharma AN, Patel BC. Laser Fitzpatrick Skin Type Recommendations. [Updated 2022 Mar 9]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK557626/>