Active.Lite®

Patent Pending Appliction Number 61/970.007

Naturally Potent + Sustainable

Rapid Skin Lightening Result in under 14 days!

evens skin tone, brightens complexion, diminishes appearance of dark spots!

BACKGROUND

Aesthetic perfection is now the consumer standard. Uneven skin tone is one of the primary visual cues revealing apparent age. Products successful in this category must provide rapid and visually perceivable results. To meet this need, Active Concepts developed **Active.Lite®**, a revolutionary, patent-pending skin lightening product.

Skin lighteners are no longer solely a province of Asia, they are now dominant in Western Markets, specifically the US and Europe. Culturally, these preparations are popular for their ability to achieve fairer, whiter-looking skin in the East but have been redefined as Western anti-aging applications, facilitating brighter, more radiant skin. The long standing aesthetic standard of Asia has transformed the cosmetic market. However, negative press surrounding skin lighteners has caused some doubt as consumer reviews reporting skin irritation and lackluster results are reported.

SCIENCE

Active Concepts is heavily invested in the study of the cross talk between micro-flora and the human body. Malassezia is a commensal organism, "but also by their implication in diseases with distinct absence of inflammation despite the heavy load (Pityriasis versicolor) or with characteristic inflammation (eg, sebborheic dermatitis, atopic dermatitis, folliculitis, or psoriasis)"[1] has been known to produce localized hypopigmentation. Modern research suggests, in essence, this yeast talks to the skin, and it is this cross talk that causes localized inhibition of melanogenesis. Based on this information, Active Concepts has done further research looking at the ability of using *Saccharomyces cerevisiae* to mimic this type of cross talking behavior associated with Malessezia.

Code Number: 22040

INCI Name: Saccharomyces/Grape Ferment Extract
INCI Status: Conforms
REACH Status: Complies
CAS Number: 84929-27-1
EINECS Number: 284-511-6

Origin: Botanical & Synthetic
Processing:
GMO Free
No Ethoxylation
No Irradiation
No Sulphonation
Additives:
Preservatives: None
Antioxidants: None
Other additives: None
Solvents Used: Butylene Glycol
Appearance: Clear to Slightly Hazy Liquid
Soluble/ Miscible: Water & Alcohol
Ecological Information:
80.00% Biodegradability
Microbial Count: <100 CFU/g, No Pathogens

Suggested Use Levels: 1.0 – 10.0%
Suggested Applications: Evens Skin Tone and Complexion, Dark Spot Treatment, Skin Lightener

Benefits of **Active.Lite®**:

- Patent Pending Skin Lightener
- Naturally Potent & Sustainable
- Lighening Results in Under 14 days
- Evens Skin Tone
- Brightens Complexion

Active.Lite®

Patent Pending Appliction Number 61/970.007

We began looking at yeast grown on different biomasses, with particular attention to the the bio-transformation of phytochemicals present. We screened these samples for their ability to inhibit melanogenesis. The product that came out the strongest was yeast based on *Vitis vinifera*. In further investigation, we looked for the mechanism of action responsible for hypopigmentation and discovered that it works through Histamine 3 Receptor Antagonism. We speculated that the amines in *Vitis vinifera* generate Histamine 3 Receptor (H3R) Antagonists.

There are four types of Histamine Receptors, two of which, Histamine Receptors 1 and 2, were previously evaluated for their ability to inhibit melanogenesis[4]. Active Concepts looked at the signaling pathway of the Histamine 3 (H3) Receptor, which prior to this, was thought to not be involved in the melanogenesis process. However, H3 receptors play a significant role in this pathway. During an H3R Transfection, we looked at melanocyte stably transfected with H3 receptors[2]. Once the test cells were transfected with H3 receptors, melan inhibition assays were conducted to support our claim that **Active.Lite®** worked via H3R Antagonism. Active Concepts' innovative mechanism of action uses the fermentation of grape biomass to shut this pathway down and stop melanogenesis.

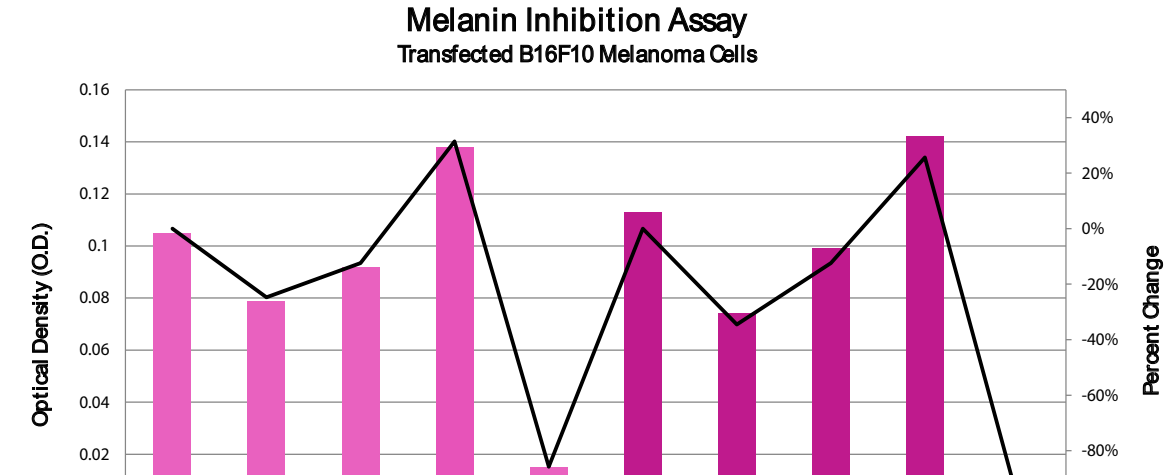
Therefore, we can infer that **Active.Lite®** represses the transcription of melanogenic genes through the inhibition of the upstream melanogenesis response. This attenuates the effect of cascading inflammatory events, thus resulting in skin lightening and similar depigmentation effects.

BENEFITS

Active.Lite® is a naturally potent, sustainable product with a patent-pending mechanism of action that produces visibly rapid lightening results in under 14 days! Demonstrated results showcase **Active.Lite®** as the product for reducing the appearance of dark spots and other hyperpigmentation on hands, face, and décolleté without irritation!

EFFICACY DATA

The Histamine 3 Receptor (H3R) gene was stably transfected into B16F10 murine melanocytes to increase H3R expression. Western blot analysis confirmed the increased expression of H3R in our transfected cells. These transfected cells were used in a Melanin Inhibition Assay to confirm **Active.Lite's®** affinity for the H3R. The increase in Melanin Inhibition in the H3R transfected cells compared to non-transfected/wild-type cells indicates that **Active.Lite®** utilizes the Histamine Receptor Signaling Pathway to Inhibit Melanin production.



	Wild Type B16F10					H3 Receptor Transfected B16F10				
O.D.	0.105	0.079	0.092	0.138	0.015	0.113	0.074	0.099	0.142	0.001
% Change	0.00%	-24.76%	-12.38%	31.43%	-85.71%	0.00%	-34.51%	-12.39%	25.66%	-99.12%

Figure 1. Melanin Inhibition Assay evaluating Transfected B16F10 Melanoma Cells.

Active.Lite®

Patent Pending Appliction Number 61/970.007

Histamine is released from the dermal mast cells when stimulated with ultraviolet radiation, which activates a cascade of inflammatory events. Inflammation in the skin can lead to hyperpigmentation through various pathways, including the initial histamine release. The histamine will further stimulate melanogenesis in melanocytes through upregulation of tyrosinase. **Active.Lite®**, created via the yeast fermentation of grape biomass, demonstrates proven skin lightening results. Comfirmed inhibition of both tyrosinase and melanogenesis are supported through *in-vivo* and *in-vitro* testing.

Arbutin, Kojic acid and AA2G are three effective, and well known, market standard skin lightening products in the cosmetic industry. However, the average time it takes to see results from these skin lighteners from initial application is approximately one month. Our product has demonstrated visible results in as little as two weeks from initial application.

Solutions of **Active.Lite®** (5%, 0.05%, 0.00005%), Kojic acid (positive control), Arbutin (positive control), L-tyrosine, and mushroom tyrosinase were prepared in 0.1M Phosphate Buffered Saline. Phosphate Buffered Saline was used as the negative control. For the inhibition assay, 10µL of test material and controls were combined with 170µL of 1mM L-tyrosine and 20µL 1000U/mL mushroom tyrosinase in a 96-well microtitre plate. The plate was placed in the Synergy H1 reader set to 37°C and optical density measurements were then taken every minute for 20 minutes at 490nm.

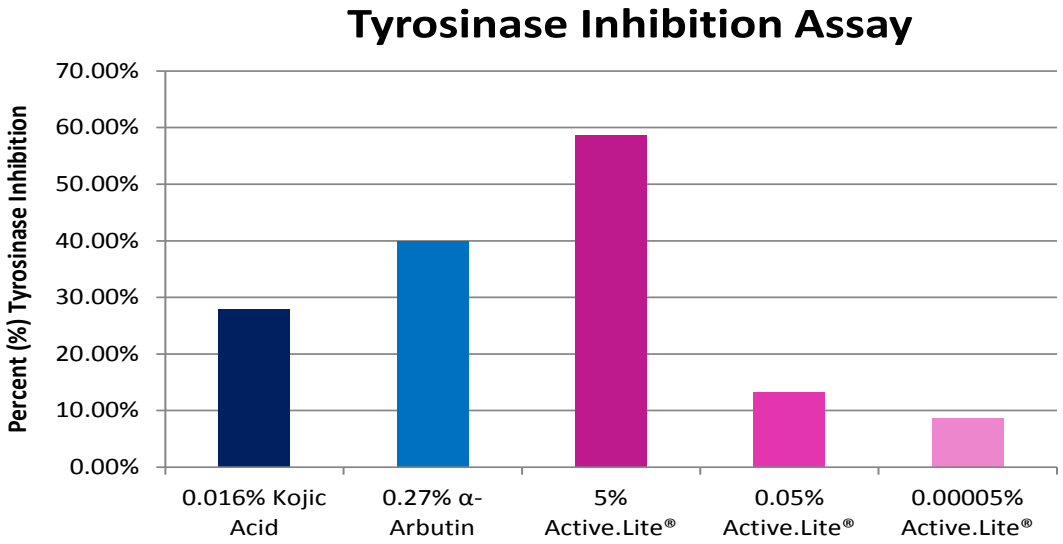


Figure 2. Tyrosinase Inhibition of skin lightening market standards in comparison to **Active.Lite®**.

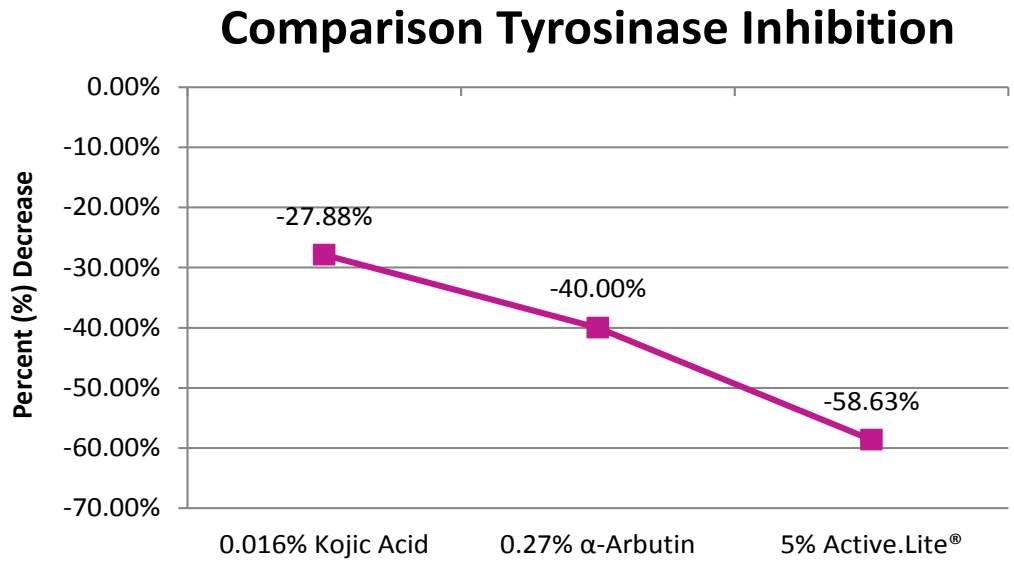


Figure 3. Comparison of positive controls Tyrosinase Inhibition to **Active.Lite®**.

The *in-vitro* Tyrosinase Inhibition assay was performed using Kojic Acid and Arbutin as positive controls to test the efficacy of **Active.Lite®**. **Active.Lite®** inhibited tyrosinase production 52.45% more effectively than Kojic Acid and 31.78% better than Arbuin. Kojic Acid and Arbutin are used as the positive control as they are market standard skin lightening ingredients in the cosmetic industry.

To further test skin lightening, we also conducted an *in-vitro* Melanin Inhibition Assay. The results of the assay are analyzed and compared to known melanin inhibitors, such as Arbutin, Kojic Acid and Ascorbic Acid 2-glucoside. B16F10 murine melanocytes were seeded into 24-well tissue culture plates and allowed to grow to confluency in complete DMEM. Solutions of **Active.Lite®**, Arbutin, Kojic Acid and AA2G were prepared in Complete DMEM. Complete DMEM was used as the untreated control.

Cells were treated for 72 hours. For the inhibition assay, the media was removed from the wells and the monolayers were washed with PBS. NaOH was added to each well and 100µL of each solution were added to a 96-well plate in duplicate. The plate was measured by optical density readings at 400nm. The greater the inhibition exhibited by the sample, the lower the optical density value due to the lack of melanin present.

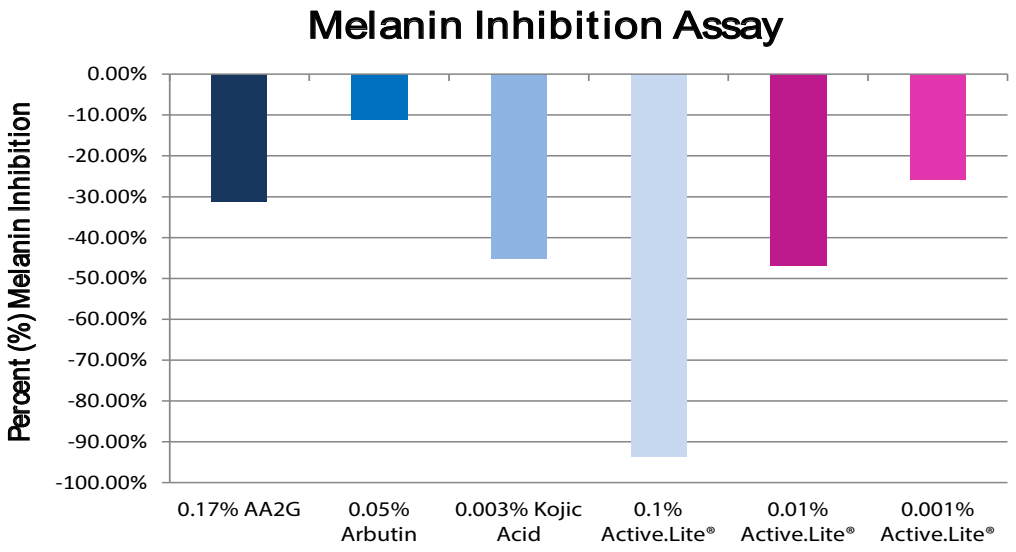


Figure 4. Melanin Inhibition of skin lightening market standards in comparison to **Active.Lite®**.

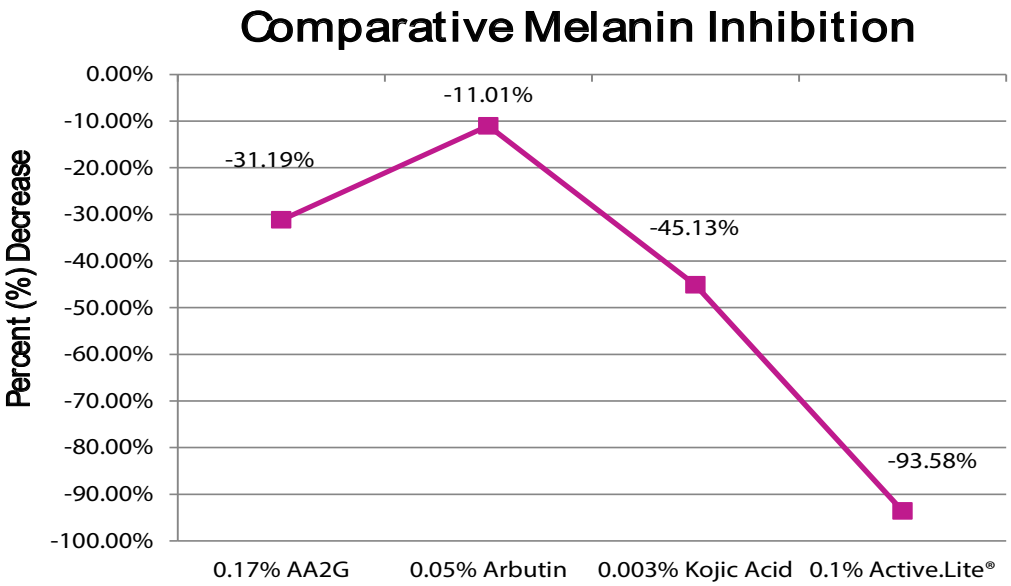


Figure 5. Positive controls for Melanin Inhibition compared to **Active.Lite®**.

Active.Lite®

Patent Pending Appliction Number 61/970.007

Looking at **Active.Lite®** ability to inhibit melanogenesis in comparison to Kojic Acid, Arbutin and AA2G . These materials are used in other cosmetic products all over the world. **Active.Lite®** was able to inhibit melanin production by 93.58%, making it 51.77% more effectively than Kojic Acid, ~88.23% better than Arbutin, and ~66.67% better than AA2G .

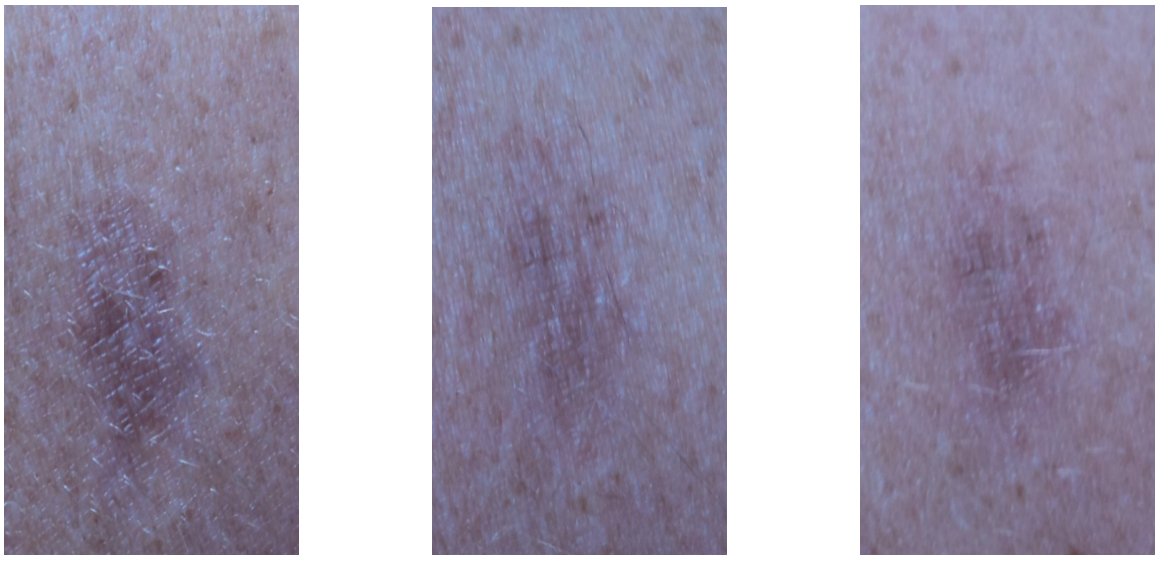
Active.Lite®, created via the yeast fermentation of grape biomass, demonstrates proven skin lightening results. Supported through *in-vivo* and in-vitro testing, **Active.Lite®** is effective in reducing of both tyrosinase and melanogenesis.

We also evaluated the ability of **Active.Lite®** to lighten skin *in-vivo*. To test this, subjects applied a lotion containing 5.0% **Active.Lite®** to their selected hyperpigmentation once a day for four weeks. Photos were taken twice a week during the four week study.

SUBJECT ONE - ACTIVE.LITE® *IN VIVO* TREATMENT

Based on the photos taken of subject one's arm, it is apparent in the initial photo that there is hyperpigmentation in the upper left frame of the image in addition to slight hyperpigmentation in the center of the image. After week two, the hyperpigmentation in the upper left portion of the frame has significantly lightened and by week four has virtually disappeared. The effectiveness of **Active.Lite®** to lighten dark spots and even skin is visibly perceivable from these photos, with rapid results appearing in less than 14 days of application.

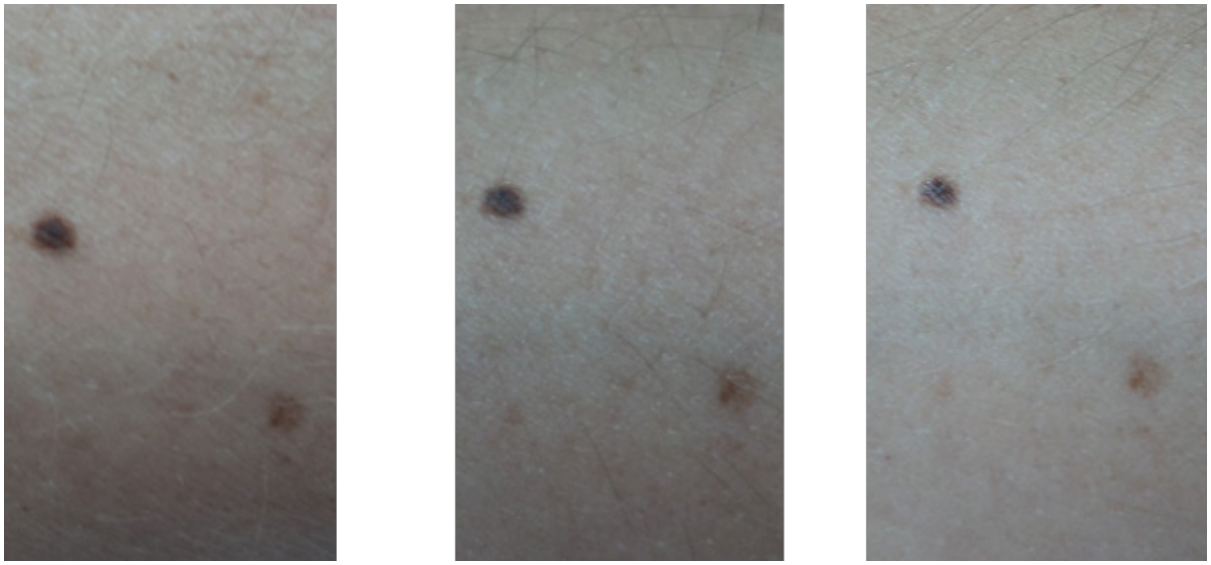
SUBJECT TWO - ACTIVE.LITE® *IN VIVO* TREATMENT



Active.Lite®

Patent Pending Appliction Number 61/970.007

SUBJECT ONE - ACTIVE.LITE® *IN VIVO* TREATMENT



Based on the photos taken of subject two's arm, it is apparent in the initial photo that there is a dark spot of hyperpigmentation in the form of a freckle/mole in the middle of the frame. After two weeks of **Active.Lite®** application to the area, the dark spot has lightened significantly and the skin has become more even. This evolution is even more pronounced in the image of the subject's arm after four weeks of **Active.Lite®** application.

Active Concepts, LLC
Lincolnton, NC. USA
www. activeconceptsllc.com
Office: +1 (704) 276 7100
info@activeconceptsllc.com

Active Concepts S.r.l.
Milano ITALY
www.activeconcepts.it
Tel +39 02 90360719
info@activeconcepts.it

Active Concepts LLC, Asia
Kaohsiung, Taiwan
www.activeconceptsllc.com
Tel + 886 73599900
josephyeh@activeconceptsllc.com