ReGeniStem™ Brightening
A Brighter Solution for Even Skin Tone

ReGeniStem™ Brightening CR
INCI Name: Glycyrrhiza glabra (Licorice) Root Extract and Prunus armeniaca (Apricot) Kernel Oil
SAP Code#: 141380

ReGeniStem™ Brightening
INCI Name: Glycyrrhiza glabra (Licorice) Root Callus Culture Extract and Prunus armeniaca (Apricot) Kernel Oil
SAP Code#: 141370

Key Product Attributes:
- Visibly brighten skin
- Enhance skin tone balance
- Target skin imperfections
- Sustainable meristematic cell culture product
- Stable, convenient, oil-based active
- Acceptable for use in China (CR version)

Background Information

First impressions are everything so having a healthy, balanced complexion is a must. It’s a challenge every person confronts, no matter their age. Imperfections come in many different forms; whether they are under-eye circles, beauty marks, or simply lackluster skin, we all have them and handle them in our own, sometimes novel, ways. ReGeniStem™ Brightening is the latest skincare active from Lonza to tackle your imperfections. Designed to brighten skin and even skin tone without irritation, it is your next solution for truly marvelous skin.

The gold standard active for addressing uneven skin tone is hydroquinone. It works by affecting pigmentation in the skin through the inhibition of tyrosinase, an enzyme responsible for melanin synthesis. Less melanin in the skin leads to a lighter skin tone and overall complexion. Hydroquinone, however, inhibits melanin synthesis wherever it is applied on the skin, potentially leading to more unbalanced skin tone compared to the original state of the treated skin. In addition, hydroquinone is a known skin sensitizer, which can lead to red, irritated skin over time.
Other popular actives to help improve skin tone balance include arbutin and kojic acid. These two actives purport to be functional equivalents to hydroquinone while reducing the negative side effects. Arbutin, a compound found in bearberry, lightens skin in a gentler manner compared to hydroquinone. Unfortunately, arbutin is a hydroquinone derivative and, over time, may potentially cause the negative side effects associated with hydroquinone. Kojic acid is a compound found in fungi. It works much like hydroquinone in that it inhibits the activity of the tyrosinase enzyme, therefore reducing the amount of melanin synthesized. Kojic acid, however, is unstable in many skin care formulations. It is prone to oxidation and reacts with sunlight, making it a challenging ingredient to formulate with.

ReGeniStem™ Brightening is Lonza’s latest skincare active for brightened, balanced skin. Derived from the meristematic stem cells of licorice, this easy to use, oil-soluble skincare active contains a high level of actives to help brighten skin. In addition, ReGeniStem™ Brightening targets skin imperfections and helps improve overall skin tone.

**Product Information**

Lonza Consumer Care is pleased to introduce our next generation biotech active, ReGeniStem™ Brightening. It is the product of our expertise in plant sciences and bio-fermentation. Meristems are tissues in plants that contain undifferentiated cells. They can be found at the shoot and the root apex of a plant. Plants are unique among living things because they are totipotent. Totipotent cells have the ability to change into any type of cell. When grown on solid culture media, meristems form calli which can then be transferred into a liquid culture media that helps maintain and sustain the undifferentiated cells, which is now called a suspension culture. It can take several months to grow a callus depending on the plant species. One of the main advantages of growing suspension cultures in bioreactors is the ability to control the growth of the plant cell and it allows Lonza to apply unique stresses to induce the production of secondary metabolites. Without stress, the undifferentiated cells do not produce appreciable quantities of secondary metabolites.

Tips of the licorice root are grown as a callus and placed on a proprietary solid plant nutrient agar. The callus is grown to a specific mass and then transferred to two different proprietary liquid plant culture mediums; a water-based and an oil-based liquid medium. The cell suspension culture is grown under tightly controlled conditions. During the active growth phase, a proprietary elicitor is added to the system to provoke expression of key secondary metabolites. The cells are grown until a final cell density is reached. The solutions are homogenized and the solids are removed. The solution is then concentrated and a suitable preservative is added to maintain the integrity of the product.

The licorice root samples are placed in a liquid suspension culture for screening, specifically for tyrosinase inhibition and melanin synthesis suppression. These screening criteria were chosen because these are the functions of hydroquinone. The conclusion of the screening process yielded the selection of an oil-based licorice callus culture as the best candidate for further evaluation and development for the ReGeniStem™ platform.

ReGeniStem™ Brightening, a Sustainable Skin Brightening Active

ReGeniStem™ Brightening is produced using a patented process that elicits secondary metabolites from meristem cultures obtained from licorice. Licorice, or *Glycyrrhiza glabra*, is native to southern Europe and parts of Asia, with Iran, Afghanistan, China, Pakistan, and Turkey actively producing the plant. Licorice has a long history, dating back to Egypt and the tomb of King Tutankhamun. To the Egyptians, licorice root was seen as a “cure-all” plant and was incorporated in many drinks to help soothe sore throats and alleviate symptoms of bronchitis.

Licorice contains many unique and complex compounds. Glycyrrhizin is one of the more well-known compounds found in licorice, which contributes to the sweet taste of the licorice root. In fact, glycyrrhizin has been found to be thirty to fifty times sweeter than refined sugars. In addition, licorice contains isoflavones such as glabrene and glabridin, the latter which has been well researched for its ability to inhibit melanogenesis and serves as the primary active in ReGeniStem™ Brightening.
The licorice starting material comes from farms native to Asia. The raw licorice grows in its natural environment and takes up to four to five years before the plant can be harvested. Lonza’s proprietary meristematic culture process differentiates ReGeniStem™ Brightening from other licorice based ingredients because the technology utilizes calli that take no longer than a month to grow. Utilization of the meristematic culture technology reduces the dependency of licorice plant harvests, providing a more uniform, reliably sourced skincare active.

In addition, many licorice-based ingredients traditionally consume the entire plant in production. ReGeniStem™ Brightening utilizes only the tips of the licorice root to grow the callus. This greatly reduces the amount of raw material needed to create ReGeniStem™ Brightening while preserving the licorice plant to be used for future product development.

**In-vitro Efficacy Studies**

**In-vitro Tanned Epidermal Melanin Assay**

ReGeniStem™ Brightening was evaluated in a tanned epidermal melanin assay, comparing its ability to inhibit melanogenesis against standard ingredients commonly found in skin lightening products. These skin lightening ingredients include hydroquinone, kojic acid, and arbutin. A water-based licorice extract was also included in the assay to compare to ReGeniStem™ Brightening.

![Graph showing melanin assay results](image)

Fig. 3 Melanin assay comparing ReGeniStem™ Brightening to common skin brightening ingredients. ReGeniStem™ Brightening showed a statistically significant change in melanin compared to untreated skin.

The results from the tanned epidermal melanin assay showed ReGeniStem™ Brightening outperformed all three of the common skin lightening ingredients and the water based licorice extract. ReGeniStem™ Brightening at 0.01% and 0.02% showed a statistically significance change in melanin compared to untreated skin.

An additional assay was performed to measure tyrosinase inhibition. The tyrosinase enzyme is a key contributor to melanin synthesis and the mechanism of focus. Inhibition of tyrosinase leads to a lighter, brighter skin tone. ReGeniStem™ Brightening was compared to two common actives known for their ability to inhibit tyrosinase activity: kojic acid and arbutin. The results show ReGeniStem™ Brightening performed comparably to kojic acid and arbutin in inhibiting tyrosinase activity.
In-vivo Efficacy Studies

In-vivo Skin Brightening Study

A preliminary 5-person, 22 day skin brightening study was initiated to evaluate the efficacy of ReGeniStem™ Brightening in reducing the color of skin. Skin was irradiated with a 2 minimal erythema dose (MED) on the back of panelists. ReGeniStem™ Brightening was incorporated into a lotion base at 0.2%, 0.4%, 1%, and 2% use levels. Kojic acid, the positive control of the study, was also incorporated into a lotion base at 0.5% and 1% use levels. The base lotion served as the placebo in the study. The lotions were applied daily on the panelist back over the 22 day study, with measurements made on Day 8 and Day 22. The treated skin was measured with a Minolta chromameter and measured for the L* values of skin color. A greater change in L* values indicates lighter skin tone.

In-vivo Skin Brightening Study with ReGeniStem™ Brightening

![Graph showing % change in L* value for in-vivo skin brightening study with ReGeniStem™ Brightening compared to kojic acid, a common skin lightening ingredient.](image-url)
The results show ReGeniStem™ Brightening at the lower concentrations (0.2%, 0.4%) performed comparably to kojic acid at 0.5%. ReGeniStem™ Brightening at the higher concentrations (1%, 2%) performed comparably to kojic acid at 1%. Based on the results, ReGeniStem™ Brightening has shown the ability to brighten the color of skin and help even skin tone. Additionally, the results justify evaluating ReGeniStem™ Brightening at a 1% and 2% use level for a larger, *in-vivo* study.

**In-vivo Skin Brightening Study**

An 84-person, 60-day study was initiated to evaluate the efficacy of ReGeniStem™ Brightening in improving skin brightening. The 84-panelist pool was divided into three 28-person groups, with each group applying one of three products on their entire face. The products were a lotion containing 1% ReGeniStem™ Brightening, a lotion containing 2% ReGeniStem™ Brightening, and a control lotion (the base lotion). Each group contained an evenly distributed population of gender and ethnicities: 25% African-American, 25% Asian, 25% Caucasian, and 25% Hispanic men and woman. Panelists applied the lotion on their face twice daily, once in the morning and once before bed. Measurements were made on Day 30 and Day 60 of the study with the Canfield VISIA CR system using visible light and analyzed using the Vaestro Image Analysis Toolkit. The skin brightening study was initiated in late spring and concluded in the middle of summer.

The results from the 84-person *in-vivo* study showed ReGeniStem™ Brightening improved skin brightening at use levels as low as 1%. The panelist from fig. 6 was measured to have a 0.55% improvement in skin brightening over baseline via Vaestro Image Analysis Toolkit, a value which, when initially read, would indicate very little improvement. However, when correlating the measurement to the images generated from the study, a 0.55% measurement does show a visible improvement in overall skin tone.
**In-vivo Skin Brightness Study with ReGeniStem™ Brightening**

Across the entire *in-vivo* study, ReGeniStem™ Brightening showed a statistically significant improvement in skin brightness of the panelist evaluated, with a mean improvement in skin brightness of 1.00% when utilizing ReGeniStem™ Brightening at a 1% use level. In addition, we see a dose dependent response in improving skin brightness, with a 1.20% improvement in skin brightness when utilizing ReGeniStem™ Brightening at a 2% use level.

**Additional *in-vitro* Data**

**In-vitro Protein Assay**

The images generated from the *in-vivo* study showed an improvement in overall skin tone, while targeting imperfections and hyperpigmentation very effectively. In addition, there are signs there are anti-aging benefits such as helping to reduce the appearance of fine lines and wrinkles. A protein assay was performed on ReGeniStem™ Brightening to discover any additional benefits which the novel active may provide the skin.

The results showed ReGeniStem™ Brightening upregulated proteins responsible for the synthesis of Collagen Type-I and Collagen Type-III. The upregulation of these collagen producing proteins could lead to anti-aging properties, such as improving skin elasticity and firming up dull, lagging skin.
Additional Clinical Images

In-vivo Skin Brightening Study – 2% ReGeniStem™ Brightening

Fig. 12
Panelist treated with 2% ReGeniStem™ Brightening with a 0.22% improvement in skin brightening over baseline via Vaestro Image Analysis Toolkit

Fig. 13
Panelist treated with 2% ReGeniStem™ Brightening with a 1.57% improvement in skin brightening over baseline via Vaestro Image Analysis Toolkit

Fig. 14
Panelist treated with 2% ReGeniStem™ Brightening with a 3.40% improvement in skin brightening over baseline via Vaestro Image Analysis Toolkit

References:

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