



Biocell SOD

Stabilized Superoxide Dismutase



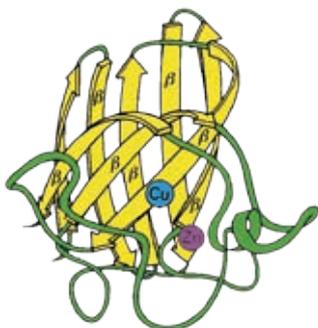
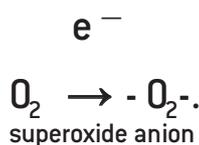
INCI Name: Superoxide Dismutase
SAP Code#: 125690

Key Product Attributes

- Reduces UV-induced erythema
- Protects against lipid peroxidation - neutralizes the superoxide radical
- Protects against damaging effects of the environment
- Enhances protective effects of sun care products
- Combats photoaging

Background Information

The formation of free radicals can be devastating to the life of a cell. Free radicals are formed by a number of different pathways, one of which is via UV radiation. Oxygen molecules, when in their ground state, are relatively harmless. However, when oxygen molecules are irradiated by UV light and thus reduced by one, two or three electrons, they become highly reactive. These highly reactive free radicals then have the ability to become extremely destructive causing mutagenesis, protein denaturation and lipid peroxidation. When cellular membranes are damaged or proteins are denatured, aging is accelerated. One such highly reactive free radical is the superoxide anion.

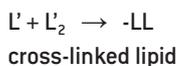
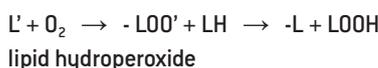
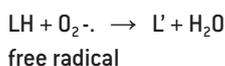


There is a line of defense though, and that is the enzyme, Superoxide Dismutase.

Product Information

Superoxide Dismutase (SOD) is an enzymatic antioxidant well known for its role in neutralizing the superoxide free radical. Developed for cosmetic use, Biocell SOD is a unique form of yeast derived, Cu/Zn protein bonded Superoxide Dismutase. It is widely believed that SOD has the ability to enhance sunscreen products and protect against the damaging effects of the environment. These effects increase the amount of free radicals the body encounters. Biocell SOD, a free radical scavenger, balances out the number of excess radicals, ultimately protecting the skin from unnecessary damage. Biocell SOD soothes the skin and helps combat against photoaging, leaving the skin looking healthy, radiant and translucent.

The formation of free radicals can lead to the formation of cross-linked lipids (L) which compromise and potentially damage cell membrane integrity.



An initiator is required to start the peroxidation chain reaction. Possible initiators include UV light, univalent oxygen, singlet oxygen and ozone. Once the reaction is under way, unless an anti-oxidant intervenes or two free radicals combine, cell destruction is inevitable. Biocell SOD is one enzyme that functions as a catalyst to quench the superoxide radical, converting it to hydrogen peroxide and molecular oxygen. Biocell SOD remains intact to continue on in its defense of the body, unlike non-enzymatic antioxidants which are consumed during the neutralization process.

Efficacy Studies

Reduction in UV-Induced Erythema

Work has been done to show that topical application of SOD will reduce the erythema elicited by UV radiation. *In vitro* studies were conducted to evaluate the efficacy of various antioxidants in reducing UV induced changes in several endpoints. The two endpoints examined here are lactate dehydrogenase (LDH) and prostaglandin E2 (PGE2). All products tested, as well as the assay kits for LDH and PGE2, were obtained from commercial sources. The studies were carried out using Human Skin Equivalents (HSEs). HSEs were obtained from commercial sources. HSEs are composed of human skin cells cultured on top of a supporting matrix. The uppermost level of tissue has an intact stratum corneum.

Lactate dehydrogenase is the enzyme responsible for the reduction of pyruvate by NADH to NAD+ and lactate. It makes an interesting marker for antioxidant studies as its activity is significantly affected by UV radiation (Figure 1).

Prostaglandin E2 is part of the arachidonic acid inflammatory cascade. Inhibition of PGE2 synthesis may be inferred to block the development of visible irritation. This theory is well born out by the *in vivo* studies conducted subsequent to this study (Figure 2).

Currently it is possible to evaluate UVB induced erythema by means of skin reflectance spectrophotometry. The use of this technique allows for the development of accurate readings in CIE Lab space. The value is that these numbers can be correlated with hemoglobin absorption curves which correlate directly to increased cutaneous blood flow. This study uses skin reflectance spectrophotometry to differentiate between the effects of various antioxidants.

For the purpose of this study the materials were provided by the noted suppliers: Tocopherol (BASF), Tocopheryl Acetate (BASF) and Ascorbyl Palmitate (Bracco). The actives were incorporated into the formulas at the following concentrations: Tocopherol (2%), Tocopheryl Acetate (2%), Ascorbyl Palmitate (1%), Biocell SOD (0.1%).

Twelve volunteers aged 24 to 40 were used for the study. The experiment was conducted at 22 ± 2°C and 40-50% relative humidity. Panelists were allowed to normalize for 15 minutes prior to measurement. A UVM-57 (UVP, San Gabriel, California) was used to elicit erythema. The minimal erythema dose was established for each panelist.

Based on those values a 2 MED dose was calculated.

Randomized sites on the central forearms were treated with 50 mg of the test products delivered by a syringe. The test sites were then occluded for 6 hours using a Hill Top Chamber (Hill Top Research Inc., Cincinnati, Ohio). After six hours the cells were removed and the sites washed to remove the test products. The sites were then exposed to a 2 MED dose of UV radiation (290-320 nm with a peak output at 302 nm – 0.80 mW/cm² as measured on skin). See Figure 3.

Lactate Dehydrogenase Activity

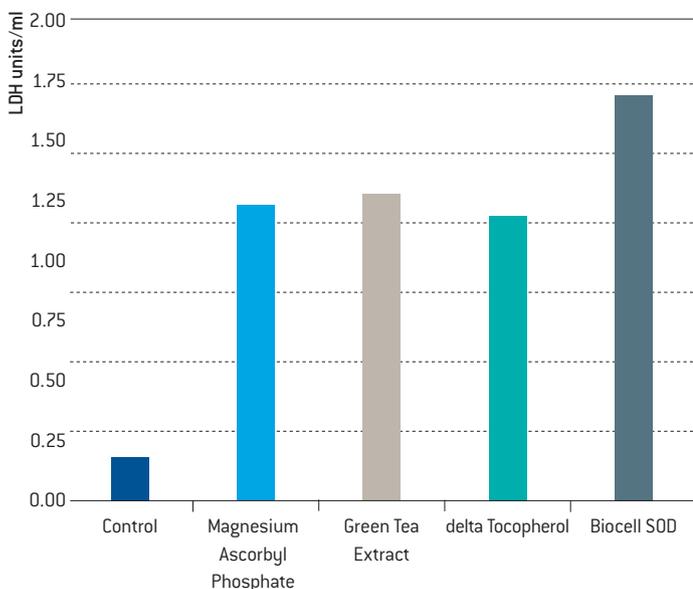


Fig 1.

Prostaglandin E₂ Synthesis

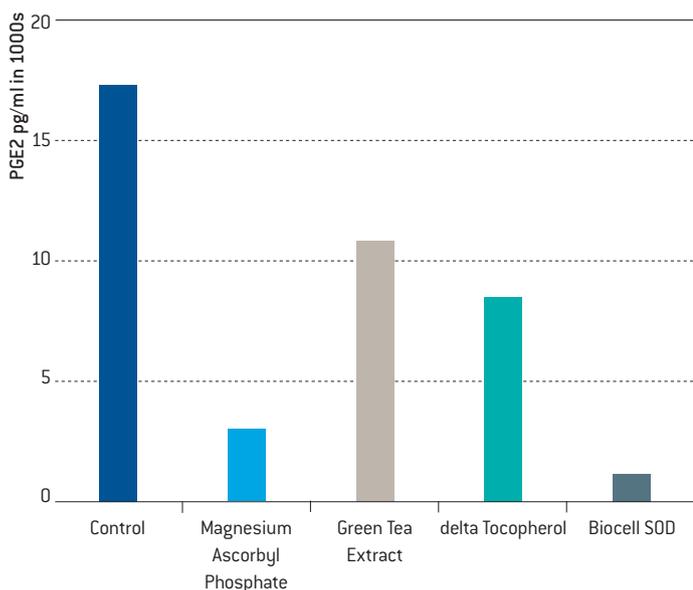


Fig 2.

Skin Reflectance Spectrophotometry

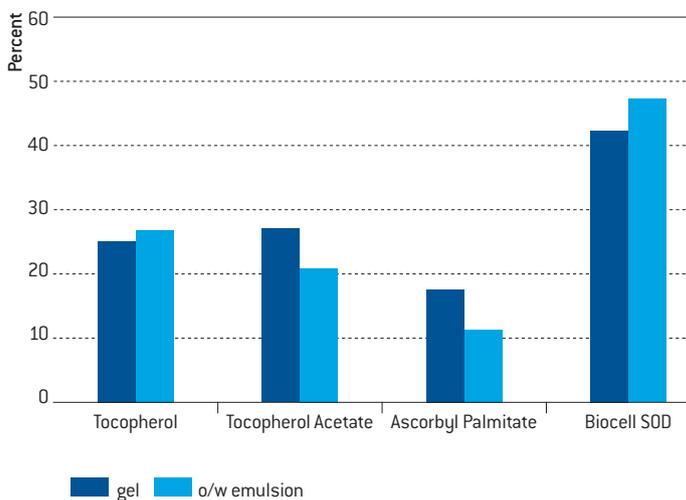


Fig 3.

The protective effect of Biocell SOD may also be enhanced by incorporation into a liposome. The study in Figure 4 was conducted to differentiate the effectiveness of Biocell SOD and Brookosome™ SOD in preventing lipid peroxidation of the skin. Brookosome™ SOD is a liposome product produced with one percent of Biocell SOD powder.

Malonyldialdehyde (MDA), a product of lipid peroxidation, levels were assessed to determine the oxidative damage to the lipid membranes. The limit of detection by this method is 0.5 nM MDA/g tissue, the nanomoles of MDA were determined based upon the absorbance. The amount of MDA produced was standardized as nanomoles of MDA per milligram of protein. The results are present in units of nM MDA/mg protein. To determine protein content, 200 ul of filtrate was added to 600 ul of H₂O and 200 ul of 5N NaOH, and boiled for 30 minutes. 100 ul of protein extract and bovine serum albumin (BSA) standards were each pipetted into labeled tubes. To each tube 2.5 ml of modified biuret reagent were added. These tubes were vortexed and then allowed to incubate for 30 minutes at room temperature. The absorbance at 540 nm was measured for each tube. A plot of BSA vs Absorbance was made. The amount of protein in each sample of homogenate was determined based upon the absorbance.

Malonyldialdehyde Synthesis

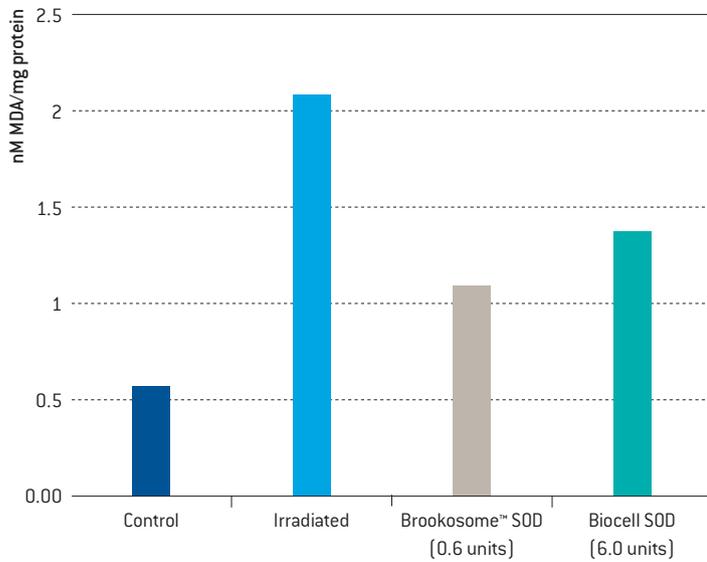


Fig 4.

Formulating Tips

Formulating with Biocell SOD and Brookosome™ SOD is quite straight forward. Biocell SOD is a water dispersible powder to be used at suggested levels of 0.1% to 0.5%. Brookosome™ SOD is liquid with an active SOD use level of 1%, thus suggested formulating use levels are 5 to 10%. Both products should be added at the end of the manufacturing procedure and at temperatures below 40°C. The optimum pH range for the final product should be 3.0 - 6.0.

USA

Lonza Consumer Care
70 Tyler Place
South Plainfield, NJ 07080
Tel +1 908 561 5200

Switzerland

Lonza Ltd
Muenchensteinerstrasse 38
4002 Basel
Tel +41 61 316 81 11

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