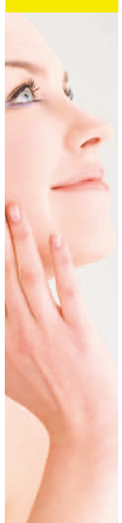
AA2G™

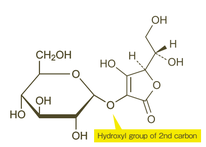


stabilized Vitamin C from Hayashibara

AA2G™

It has been known for many years that the use of vitamin C (ascorbic acid) can result in brighter, healthier, younger looking skin. Science has discovered that this lightening effect is related to the ability of vitamin C to suppress melanin synthesis and reduce existing melanin. The challenge with using vitamin C in health and beauty formulations is the fact that it can easily lose its biologic activity because of heat, oxidation, and reactions with metal ions and other common cosmetic ingredients. This causes discoloration in the cosmetic formulations and dramatically decreases the ability of vitamin C to provide the benefits of younger looking healthier skin. To overcome these problems, Hayashibara partnered with a renowned cosmetic chemist to develop a new substance consisting vitamin C and glucose using a novel enzyme manufacturing process. This has resulted in a unique stabilized vitamin C called L-Ascorbic Acid 2-Glucoside (AA-2G). This vitamin C derivative, which is now being sold under the trade name AA2G™, has superior formulation stability that essentially resists discoloration and degradation, while retaining all the biologic activity that provides lightening, UV damage and anti-aging properties.

Structure of L-Ascorbic Acid 2-Glucoside



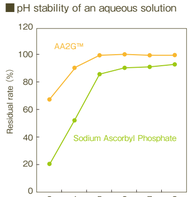
The hydroxyl group of the second carbon in vitamin C is highly reactive and plays an important role in the biologic activity, however, it can also be the site of inactivation and decomposition. L-Ascorbic Acid 2-Glucoside is produced from vitamin C and starch by use of enzymes that reversibly bind glucose to the reactive hydroxyl group to protect the site from inactivation.

Property 1

High stability

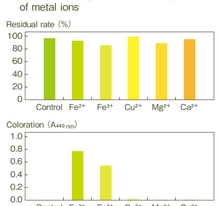
AA2G™ is highly stable in solution even at high temperatures, low pH and in the presence of metal ions. This results in AA2G™ helping to maintain product quality.

■ pH stability of an aqueous solution



Solutions of 0.2% (w/v) AA2G™ and 0.2% (w/v) Sodium Ascorbyl Phosphate were adjusted to various pH levels and filter sterilized. Each sample was then poured into an airtight glass bottle and placed in the dark at 50℃ for 20 days.

■ AA2G™ stability and color in the presence of metal ions



Five different metal salts (sulfates;10 mmol/L) were added to a solution of 0.5% (w/v) AA2G™. The pH was adjusted to 6 and the solutions were heated at 100℃ for 2.5 hours.

Property 2

Resistant to discoloration

AA2G™ is resistant to discoloration during production or storage of cosmetic products. This results in AA2G™ helping to maintain product quality.

■ Color of AA2G™ and other vitamin C derivative solutions

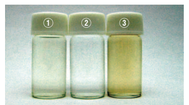


Image of 10% aqueous solutions of ①Magnesium Ascorbyl Phosphate ②AA2G™ and ③Sodium Ascorbyl Phosphate.

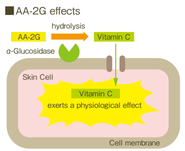
Mechanism

Mechanism of action

An enzyme releases vitamin C from AA-2G at the cellular surface and provides benefits over a sustained period of time.

AA-2G is hydrolyzed by α-glucosidase, an enzyme present in the membrane of skin cells. This process releases vitamin C in its active form. When vitamin C enters cells it results in significant and well-documented biological responses. AA-2G is hydrolyzed over a prolonged period, resulting in consistent and sustained beneficial physiological effect on the skin.

■ AA-2G effects



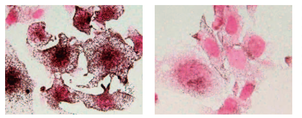
Efficacy 1

Lightens skin

Suppresses melanin synthesis

When AA-2G is converted to vitamin C and enters skin cells, it significantly reduces the production of melanin by inhibiting tyrosinase activity. This results in a reduction of dopaquinone, an intermediate of melanin synthesis. Additionally it converts existing dopaquinone to dopa.

■ Prevents melanin synthesis



Without AA-2G | With AA-2G

B16 melanoma cells were cultured for 1 day, and the media was replaced with fresh media with or without 10 mmol/AA-2G. After cultivation for 2 more days, cells were incubated for 1 hour with 0.1%(w/v) L-Dopa solution. Nuclear Fast Red was used for counter staining.

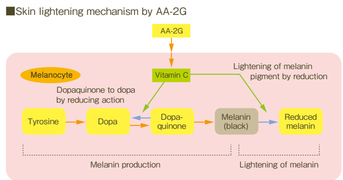
Efficacy 2

Lightens skin

Reduces existing melanin

When AA-2G is converted to vitamin C and enters skin cells, it can significantly lighten existing black melanin by a reduction reaction.

■ Skin lightening mechanism by AA-2G



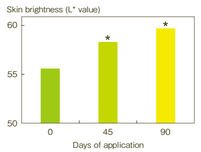
Efficacy 3

Lightens skin

Reduces skin hyperpigmentation

Creams containing AA2G™ are effective at lightening skin hyperpigmentation.

■ Measurement of skin hyperpigmentation



Sixteen female volunteers (37 to 55 years old) applied a cream containing 2% of AA2G™ twice a day to a hyperpigmented area of their faces for a total of 90 days. The areas were matched against pantone color samples and the brightness (L* value) was measured by a colorimeter on days 0, 45, and 90. *Denotes significantly different from day 0 at $p < 0.01$.

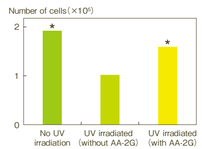
Efficacy 4

Protects from UV damage

Suppresses damage to cells caused by UV irradiation

When AA-2G is converted to vitamin C, it can dramatically reduce the free radicals that result from UV irradiation of the skin, thereby significantly reducing cell damage.

■ Inhibits UV damage to skin fibroblasts



Human skin fibroblasts were cultured for 1 day. Fibroblast samples were cultured a second day with or without 1 mmol/L AA-2G. The cells were irradiated with 20mJ/cm² UVA and B radiation, and then cultured for 2 additional days. The numbers of viable cells were determined. *Denotes significantly deferent from UV irradiated cells without AA-2G at $p < 0.05$.

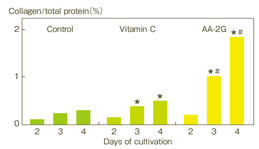
Efficacy 5

Anti-aging

Promotes collagen synthesis

Collagen is a protein that plays an important role in the structure and firmness of skin. When AA-2G is converted to vitamin C and enters skin cells, it promotes collagen synthesis of skin fibroblasts. AA-2G maintains its effects for a sustained period of time.

■ Enhances collagen synthesis of skin fibroblasts



After cultivation of normal human skin fibroblast for 1 day, the cells were cultured in the presence of vitamin C (0.2 mmol/L) or AA-2G (0.2 mmol/L) for 2-4 days. The percentage of collagen to total protein was determined. *Denotes significantly different from control at $p < 0.05$, and #denotes significantly different from vitamin C at $p < 0.05$.

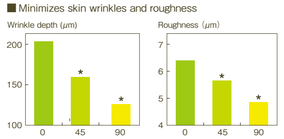
Efficacy 6

Anti-aging

Improves wrinkles and roughness

Formulations containing AA-2G™ are highly effective at reducing wrinkles and reducing skin roughness.

■ Minimizes skin wrinkles and roughness



Sixteen female volunteers (37 to 55 years old) applied a cream containing 2% of AA2G™ twice a day for a total of 90 days to the outer corners of their eyes and cheek area. Silicone replicas of the corner of the eyes and the other treated areas were taken on days 0, 45 and 90 and wrinkle depth and skin roughness were measured by profilometry. *Denotes significantly different from day 0 at $p < 0.01$.