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stabilized vitamin C from Hayashibara



for healthier, brighter, younger looking skin

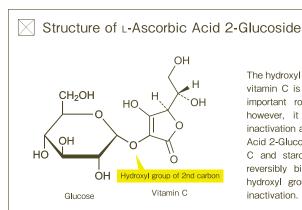






# stabilized Vitamin C from

t 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. 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.



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.



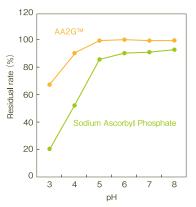
# High stability

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

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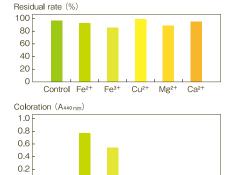
Control Fe2+

### 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°C 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°C for 2.5 hours.

Cu2+

Mg<sup>2+</sup>

Fe3+

# Hayashibara

# 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<sup>™</sup> and other vitamin C derivative solutions



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

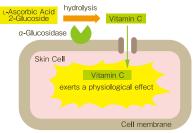


# Mechanism of action

An enzyme releases vitamin C from L-Ascorbic Acid 2-Glucoside at the cellular surface and provides benefits over a sustained period of time.

L-Ascorbic Acid 2-Glucoside is hydrolyzed by  $\alpha$ -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. L-Ascorbic Acid 2-Glucoside is hydrolyzed over a prolonged period, resulting in consistent and sustained beneficial physiological effect on the skin.

# ■ L-Ascorbic Acid 2-Glucoside effects

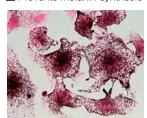




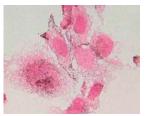
# **Lightens skin**Suppresses melanin synthesis

When L-Ascorbic Acid 2-Glucoside 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 L-Ascorbic Acid 2-Glucoside



With L-Ascorbic Acid 2-Glucoside

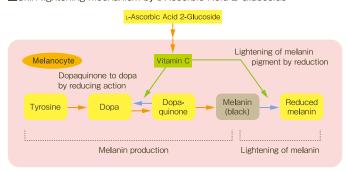
B16 melanoma cells were cultured for 1 day, and the media was replaced with fresh media with or without 10 mmol/L L-Ascorbic Acid 2-Glucoside. After cultivation for 2 more days, cells were incubated for 1 hour with 0.1%(w/v) L-Dopa solution. Nuclear Fast Red was used for counter staining.



# **Lightens skin**Reduces existing melanin

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

## ■Skin lightening mechanism by L-Ascorbic Acid 2-Glucoside





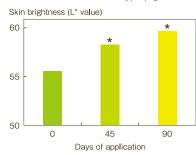
Efficacy

3

# **Lightens skin**Reduces skin hyperpigmentation

Creams containing AA2G  $^{\text{\tiny{TM}}}$  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.

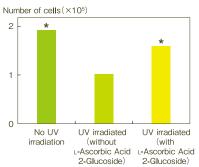


# **Protects from UV damage**

# Suppresses damage to cells caused by UV irradiation

When L-Ascorbic Acid 2-Glucoside 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



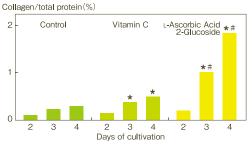
Human skin fibroblasts were cultured for 1 day. Fibroblast samples were cultured a second day with or without 1 mmol/L L-Ascorbic Acid 2-Glucoside. The cells were irradiated with 20mJ/cm² UVA and B radiation, and then cultured for 2 additional days. The numbers of viable cells were determined. \*Denotes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



# Anti-aging Promotes collagen synthesis

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

## ■ Enhances collagen synthesis of skin fibroblasts



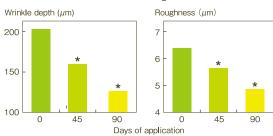
After cultivation of normal human skin fibroblast for 1 day, the cells were cultured in the presence of vitamin C (0.2 mmol/L) or L-Ascorbic Acid 2-Glucoside (0.2 mmol/L) for 2-4 days. The percentage of collagen to total protein was determined. \* Denotes significantly different from control at p<0.05,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

# Efficacy

# Anti-aging Improves wrinkles and roughness

Formulations containing AA2G<sup>™</sup> 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<sup>TM</sup> 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  $\rho$ <0.01.

# Hayashibara



## ■ AA2G<sup>™</sup> appearance and packaging





10 kg package (A tin-free steel can)

## ■ Japanese regulatory approval

Quasi-drugs containing  $AA2G^{\mathsf{TM}}$  as an active ingredient are approved in Japan. The claims are listed as: "Preventing pigmentation in age spots and freckles from sunlight" or "Suppressing melanin synthesis and preventing pigmentation in age spots and freckles." If you need further information or technical support, please feel free to contact a company representative at the number listed on the back page.

INCI Name: Ascorbyl Glucoside

AA2G is a trademark of Hayashibara and a registered trademark of Hayashibara in Japan, the U.S., Australia, Brazil, Canada, China, European Union, Hong Kong, India, Malaysia, Mexico, Philippines, Republic of Korea, Russian Federation, Singapore, South Africa, Taiwan, United Arab Emirates and Viet Nam (as of June 30, 2016).

Tips for AA2G™ formulation - to enhance the stability of your formulation -

## ■ How to use AA2G<sup>™</sup> in your formulations:

- After preparing an AA2G™ solution, neutralize it by using an alkaline agent before adding it to the formulation.
- If the formulation needs heating, cool it below 40°C before adding neutralized AA2G™ solution. Avoid heating formulations containing AA2G™ above 40°C for prolonged periods.
- Adding buffers, chelating agents and antioxidants, and shielding the product from strong light are also useful in preventing AA2G™ from decomposition during formulation.
- The stability of AA2G™ is influenced by pH. Avoid leaving it under prolonged conditions of strong acidity or alkalinity (pH 2-4 or 9-12).

## Compatibility with water-soluble polymers

If water-soluble polymers are used with AA2G™, it is recommended that they are either natural or salt-tolerant synthetic polymers.

Further technical assistance can be provided upon request.

#### Contact Us

#### **Japan**

#### HAYASHIBARA CO., LTD.

Nihon-Seimei Okayama Bldg. II Shinkan
1-1-3 Shimoishii, Kita-ku, Okayama 700-0907, Japan
(TEL/FAX) +81-86-224-4312/+81-86-233-2265
Please visit our website at http://www.intl.hayashibara.co.jp/
for general information and inquiries.

#### North and South America

#### Nagase America Corp.

546 Fifth Avenue 16th Floor, New York, NY 10036-5000, U.S.A. (TEL/FAX) +1-212-703-1340/+1-212-398-0687 havashibara@nagase-nam.com

### Europe

#### Nagase (Europa) GmbH

Immermannstrasse 65C, 40210 Dusseldorf, Germany (TEL/FAX) +49-211-866200/+49-211-3237068 hayashibara@nagase.de

#### Asia

# Nagase Business Management and Planning (Shanghai) Co., Ltd.

02, 7F Eco City,1788 Nanjing Road(W), Shanghai, P.R. China 200040 (TEL/FAX) +86-(21)33607851/+86-(21)33607852 hayashibara@nagase.cn

#### Nagase (Taiwan) Co., Ltd.

16F, No. 248, Sec.3, Nanking E.Rd., Taipei, Taiwan, R.O.C. (TEL/FAX) +886-(2)27733668/+886-(2)27737288

### South East Asia and Oceania

### Nagase Singapore (Pte) Ltd.

600 North Bridge Road #11-01 Parkview Square Singapore 188778 (TEL/FAX) +65-63980088/+65-63980227 hayashibara@nagase.com.sg

## Nagase India Private Ltd.

312, Hubtown Solaris, N.S. Phadke Marg, Near East West Flyover, Andheri East, Mumbai 400 069, India (TEL/FAX) +91-22-6681-8585/+91-22-6681-8508 hayashibara@nagase.co.in

#### NAGASE & CO., LTD.

Hayashibara Products Sec.

Personal Care Products Div. Life & Healthcare Products Dept. 5-1, Nihonbashi-Kobunacho, Chuo-ku, Tokyo 103-8355, Japan (TEL/FAX) +81-3-3665-3864/+81-3-3665-3131 dnpchb@nagase.co.jp

#### Nagase do Brasil Comércio de Produtos Químicos Ltda.

Rua Cubatão, 86, 304 e 306, Vila Mariana CEP 04013-000, São Paulo, SP (TEL/FAX) +55-11-3251-3111/+55-11-3262-3451 contato@nagasebrasil.com.br

#### Nagase (Europa) GmbH, London Branch

33 Cavendish Square, London, W1G 0PW, UK (TEL/FAX) +44-(020)7182-4948/+44-(020)3402-2427 hayashibara@nagase.de

#### Nagase (Hong Kong) Ltd.

Suites 3901 & 3912-14, Tower 6, The Gateway, Harbour City, 9 Canton Road, Tsim Sha Tsui, Kowloon, Hong Kong, S.A.R., P.R. China (TEL/FAX) +852-2302-7607/+852-2377-2720 nag.color-img-web@nagase.com.hk

#### Nagase Korea Corp.

Danam Bldg., 23F. 10, Sowol-ro, Jung-gu, Seoul, Korea 04527 (TEL/FAX) +82-(2)7538589/+82-(2)7348747

### Nagase (Thailand) Co., Ltd.

No 952, Ramaland Building, 14th Floor, Rama IV Road, Kwaeng Suriyawongse, Khet Bangrak, Bangkok 10500 Thailand (TEL/FAX) +66-(2)6327253/+66-(2)6327254 hayashibara@nagase.co.th

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INCI name: Ascorbyl Glucoside

AA2G™ is manufactured by Hayashibara Co., Ltd. in Japan.

