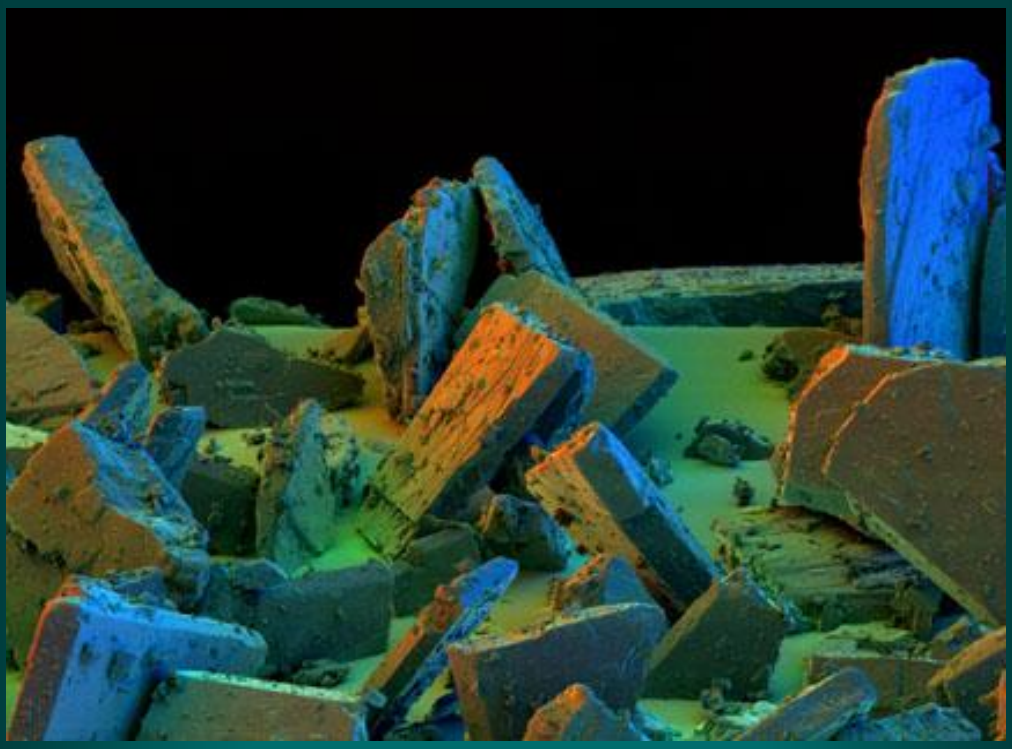
ENZYMES COUPLED ASCORBIC ACID LIQUID & POWDER



novel functional ingredients for multi-purpose formulations

CAMPO RESEARCH PTE LTD
Level 30, 6 Battery Road, Singapore 049909
Tel: (65) 63833203 / 202 / 63833631 Direct Fax (65) 63833632 / 63834034
Email: sales@campo-research.com Website: http///www.campo-research.com

CAMPO® Multi-Purpose Cosmetic Base Chemicals & Active Ingredients
CAMPO® Novel Functional Active Cosmetic Ingredient & Raw Materials

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Ask about our Herbal Natural Products Chemistry Consultancy Services – Product Registration EEC/UK New Drug Development (NDA-US); Quasi-Drug Topicals (MOHW_Japan); Development of Standards, Analysis & Profiles of Phytochemicals; Literature searches, Cultivation of Medicinal Plants, Clinical-Trials, Development of new uses for Phytochemicals and Extracts; Contract Research and Development Work in Natural Products for Novel Drugs, New Cosmetic Active Ingredients for Active Topica/OTC Cosmetic with functionality and Consumer-perceivable immediate-results, New Food Ingredients for Nutraceuticals & Functional Foods.

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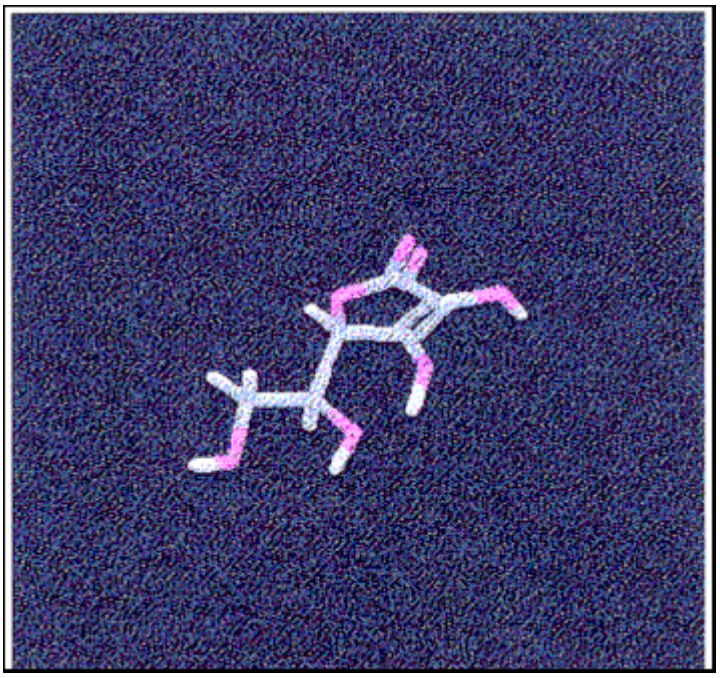
Overview

Campo Enzymes Coupled Ascorbic Acid Liquid represents an advanced way to add the benefits of ascorbic acid to personal care products. The antioxidant properties of ascorbic acid have been improved. Campo Enzymes Coupled Ascorbic Acid Liquid is a more potent collagen synthesis stimulator than ascorbic acid. Since Campo Enzymes Coupled Ascorbic Acid Liquid is a polyamine complex, the ascorbic acid portion of the complex is protected and the antioxidant and anti-collagenase properties are prolonged in finished products.

Campo Enzymes Coupled Ascorbic Acid Liquid is water soluble and can be easily incorporated into cationic and acidic nonionic emulsions. No special processing considerations are necessary as Campo Enzymes Coupled Ascorbic Acid Liquid tolerates normal emulsion processing techniques and temperatures. Recommended use levels are 1-3 percent.

Molecular Structure:

Campo Enzymes Coupled Ascorbic Liquid have found tremendous application in the personal care industy for their ability to promote collagen synthesis and to prevent oxidative damage.



Collagen Synthesis Stimulation

Ascorbic acid is essential for collagen synthesis as is evidenced by connective tissue disorders, such as scurvy, where ascorbic acid is deficient. Ascorbic acid's original importance in collagen synthesis was found to be as a co-factor for the hydroxylation of proline to create hydroxy-proline that in turn is important in collagen helix formation. It has been shown that ascorbic acid has a primary stimulatory role in collagen synthesis unrelated to hydroxylation and originating at the messenger RNA level. We have compared ascorbic acid with Campo Enzymes Coupled Ascorbic Acid Liquid for collagen stimulation.

Normal human dermal fibroblasts were cultured for 72-96 hours in the presence of ascorbic acid or Campo Enzymes Coupled Ascorbic Acid Liquid, each at 0.1% concentration. After incubation, the supernatant was removed and both supernatant and cells were assayed for collagen stimulation. A proprietary collagen ELISA test was used to quantify changes in collagen synthesis. A standard curve of collagen Types I & III was used to quantify collagen levels. Monoclonal antibodies specific for Types I & III collagen are added to the cells and to the supernatant, followed by an alkaline phosphatase-labeled secondary antibody. The levels of collagen are measured by adding a substrate for alkaline phosphatase that produces a colored product. The level of color intensity is directly proportional to the amount of collagen Types I & III present. The difference in color intensity is compared to control, untreated cells to determine relative changes in collagen synthesis.

The Campo Enzymes Coupled Ascorbic Acid Liquid is able to increase collagen synthesis by 62% in human dermal fibroblasts, an 11% increase over ascorbic acid's collagen promoting activity alone.

Inhibition of Collagenase Activity

Collagenase is a destructive enzyme secreted by cells to degrade the extra-cellular matrix protein, collagen, thus weakening the primary support structure of the skin. An enzyme substrate, (2-furanacryloyl-L-leucylglycyl-L-prolyl-L-alanine), is cleaved by collagenase resulting in a decrease in optical density (OD). The rate of change of OD is proportional to the activity and/or concentration of the enzyme present. We can measure the rate of decrease of OD over time (2-5 mins) which translates into the rate of activity. In the absence of an enzyme inhibitor, this rate is 100%. The rate of enzyme activity in the presence of various concentrations of an inhibitor can be measured and expressed as a percentage of the uninhibited control.

Figure 1 shows various concentrations of Campo Enzymes Coupled Ascorbic Acid Liquid mixed with a fixed concentration of Collagenase (TypeI). The rate of the enzyme-catalyzed reaction was measured. In the absence of Campo Enzymes Coupled Ascorbic Acid Liquid, the enzyme rate was 0.044 min^{-1}, and this was set to 100%. The concentration of Campo Enzymes Coupled Ascorbic Acid Liquid at which the enzyme was inhibited by 50% was 0.27%. At concentrations

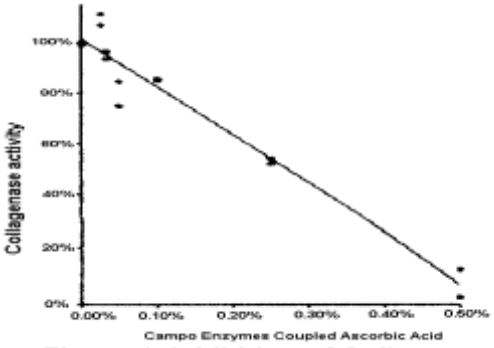


Figure 1. Inhibition of Collagenase Activity

of Campo Enzymes Coupled Ascorbic Acid Liquid above 1%, the enzyme was completely inactive, demonstrating that Campo Enzymes Coupled Ascorbic Acid Liquid is a potent inhibitor of Collagenase.

Antioxidant Activity

Free radicals derived from molecular oxygen are generated in tissues from environmental assaults. Excitation of phagocytic cells (macrophages, neutrophils, lymphocytes) results in respiratory burst, the generation of reactive oxygen species. Superoxide anion is formed during such a respiratory burst and is the precursor to a series of lethal oxidants such as hydrogen peroxide, hydroxyl radicals and hypochlorus acid. Free radicals that are not kept in check contribute to the degradation of the extracellular matrix, lipid peroxidation, hydrolyzation of polysaccharides and denaturation of proteins, leading to inflammation and tissue damage.

Cells have a highly evolved defense system in place to limit and repair the chemical damage that reactive oxygen species can cause. Enzymes such as superoxide dismutase participate in these systems. As the body ages, the natural free radical scavenging systems decline, increasing the chance for tissue damage by reactive oxygen species.

We have compared several antioxidants against Campo Enzymes Coupled Ascorbic Acid Liquid using three different in vitro assays. The malondialdehyde assay (MDA) was used to evaluate the ability of an antioxidant to protect unsaturated lipids from oxidation caused by UV light. The Cytochrome C Reduction assay is widely used for monitoring increased extracellular superoxide anion production from a respiratory burst. Intracellular reactive oxygen species can be measured using the conversion of dichlorodihydrofluorescein diacetate to the fluorescent dichlorofluorecein.

The MDA and Cytochrome C assays measure extracellular episodes where the test compound can interact freely with the event(s) occurring. Since the DCFH assay measures intracellular episodes where certain test compounds may not have access to the intracellular compartment, the assay of a test material's antioxidant efficacy is underestimated. The MDA and Cytochrome C Reduction assays provide the truest reflection of a product's antioxidant activity.

Malondialdehyde Assay

Liposomes are prepared from unsaturated phospholipids, with or without added antioxidants. The liposome preparations are exposed to UVC radiation over three hours. During irradiation, aliquots are removed to determine the amount of peroxides formed. Lipid peroxidation is determined by reacting the aliquots with thiobarbituric acid. The reaction product of malondialdehyde (MDA) is measured spectrophotometrically. This assay allows us to test both water soluble as well as oil soluble antioxidants. As shown in Figure 2, Campo Enzymes Coupled Ascorbic Acid Liquid prevents 65% of UV-induced lipid peroxidation.

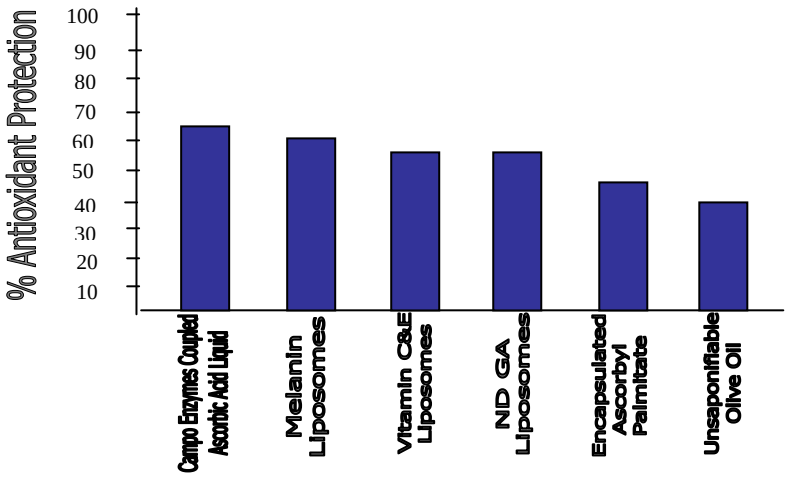


Figure 2. **Malondialdehyde Assay**
The percent antioxidant activity is calculated relative to exposed lipids without any "antioxidant" present.

Cytochrome C Reduction Assay

Primary human neutrophils are isolated from fresh blood samples, incubated with Cytochrome C, ± phorbol myristyl acetate (PMA) and the test antioxidant. When the neutrophils are irritated by the phorbol they produce a respiratory burst, secreting superoxide anion. The superoxide anion reduces the Cytochrome C which is measured spectrophotometrically over time. In the presence of an antioxidant, the radicals are captured, thus decreasing the amount of Cytochrome C being reduced. As shown in Figures 3 and 4, Campo Enzymes Coupled Ascorbic Acid Liquid is a very potent antioxidant.

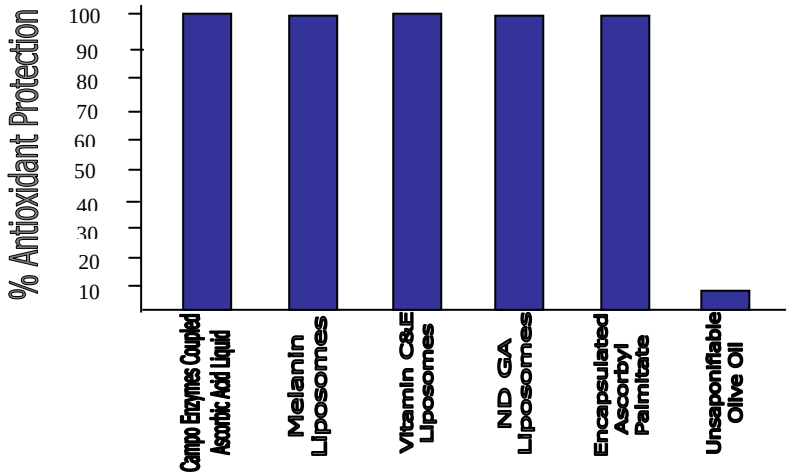


Figure 3. Cytochrome C Reduction Assay
The percent antioxidant protection is calculated relative to the PMA control. PMA will maximally stimulate neutrophils to initiate a respiratory burst, then the decrease in Cytochrome C reduction or DCFH reduction is compared relative to PMA only activated cells.

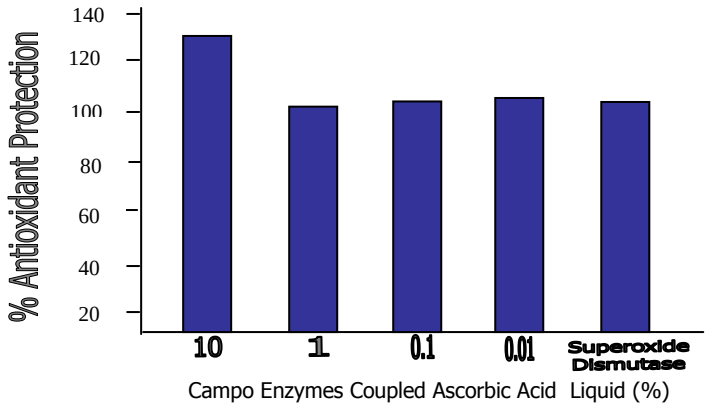


Figure 4. Dose Response of Campo Enzymes Coupled Ascorbic Acid Liquid in Cytochrome C Reduction Assay
Superoxide dismutase quenches free radicals produced by activated neutrophils.

Dichlorofluorescein Reduction (DCFH) Assay

Primary human neutrophils are incubated in the presence of dichlorodihydrofluorescein diacetate ester, a cell permeable dye. The cells are pelleted and resuspended in buffered saline to remove any unincorporated dye, then incubated ± PMA and the test antioxidant. When the dichlorodihydrofluorescein diacetate is oxidized in the presence of superoxide anion the ester is cleaved and converted to dichlorofluorescein, which is membrane impermeant and fluorescent. The amount of fluorescence can then be measured with a fluorimeter. In the presence of antioxidants, the release of reactive oxygen species from the neutrophils is reduced, thus decreasing the oxidation of the dichlorodihydrofluorescein ester. In contrast to the Cytochrome C Reduction assay, the DCHF assay measures intracellular respiratory burst. Campo Enzymes Coupled Ascorbic Acid Liquid prevents 100% of the respiratory burst (Figures 5 and 6).

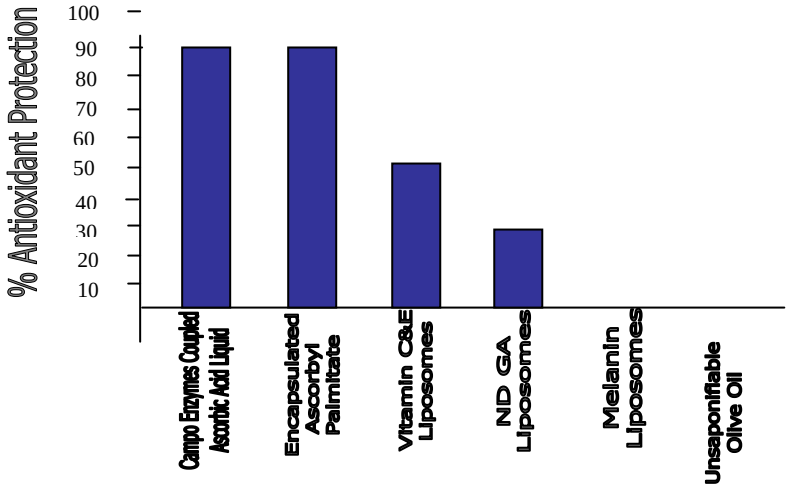


Figure 5. DCFH Assay

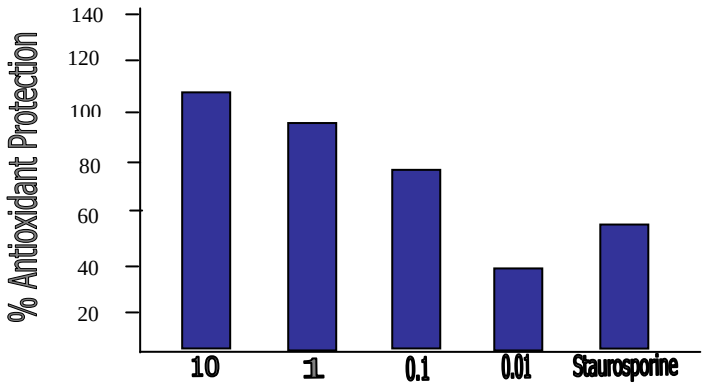


Figure 8. Dose Response of Campo Enzymes Coupled Ascorbic Acid Liquid in DCFH Assay
Staurosporine inhibits kinase c activation, thus inhibiting the initiation of the respiratory burst.

Rank Ordering of Antioxidant Activity by Assay

(%) percentage antioxidant protection

MDA	Cytochrome C	DCFH
Campo Enzymes Coupled Ascorbic Acid Liquid (65%)	Campo Enzymes Coupled Ascorbic Acid Liquid (100%)	Campo Enzymes Coupled Ascorbic Acid Liquid (90%)
Melanin Liposomes (60%)	Melanin Liposomes (100%)	Encapsulated Ascorbyl Palmitate (90%)
Vitamin C&E Liposomes (50%)	Vitamin C&E Liposomes (100%)	Vitamin C&E Liposomes (50%)
NDGA Liposomes (50%)	NDGA Liposomes (100%)	NDGA Liposomes (25%)
Encapsulated Ascorbyl Palmitate (40%)	Encapsulated Ascorbyl Palmitate (100%)	Melanin Liposomes (0%)
Unsaponifiable Olive Oil (30%)	Unsaponifiable Olive Oil (5%)	Unsaponifiable Olive Oil (0%)

All samples tested at 1% except where noted for dose response curves.
Levels of active within the liposomes:
Vit C = 0.2% / E = 1%; Melanin = 5%; NDGA = 0.2%; encap. ascorbyl palmitate (1.7% encap)
Campo Enzymes Coupled Ascorbic Acid Liquid = 20% ascorbate.
Unsaponifiable Olive Oil is not water soluble, therefore a surfactant-free dispersion of 30% oil was made to adapt it to the Cytochrome C and DCFH assays.

Typical Properties

Campo Enzymes Coupled Ascorbic Acid Liquid Typical Properties	
Physical Appearance:	Slightly viscous, clear liquid
Color:	Light yellow
pH:	2.00 – 4.00
Specific Gravity:	1.01 – 1.150
Microbiology:	
Bacteria and fungi	< 100 organisms/gram
Pathogenic	None
Comments:	A change of colour from light almost colourless liquid to dark yellow liquid may be observed upon prolonged standing. However, this change does not cause any change on its functionality. Store in cool dark room and keep bottles tightly closed.

Sample Formulary:

Hand & Body Lotion with Campo Enzymes Coupled Ascorbic Acid Liquid		
Phase	**Ingredient**	**%**
A.	Campo Enzymes Coupled Ascorbic Acid Liquid	2.00
	Deionized water	74.0
	Sorbitol 70%	5.00
B.	Campo Muruity-Muruity Oil	8.00
	Glyceryl Stearate (and) PEG-100 Stearate	2.60
	Dimethicone	0.70
	Ceteareth-20 (and) Cetearyl alcohol	1.20
	Stearyl alcohol	0.70
	Cetyl Alcohol	0.50
	Ceteth-2	0.80
	PEG-100 Stearate	0.60
	Ceterayl Alcohol	1.70
C.	Campo Plantservative WS	2.20

Procedure:
Propeller mix and heat Phase A to 75°C. Separately heat and mix Phase B to 75°C. Add Phase B into Phase A; hold temperature and mixing for 30 minutes then mix and force cool to 40°C. Add Phase C, mix until uniform. Mix and force cool to 25°C.

Skin Whitening cream (This whitening cream formulation is a suitable Novel alternative subsitute to replace any class of AHA's based creams without any AHA's.)		
Ingredient	**321418-C1179 CLQ**	**2840-C1420 CLQ**
	%	**%**
Ceramide Oil and Vegetal Lanolin Alcohol	7.00	7.00
Cetearyl alcohol	3.00	3.00
Glyceryl stearate	10.00	-
Glyceryl stearate and PEG-100 Stearate	-	10.00
PEG-40-castor oil	2.00	-
DEA-Oleth-3-phosphate	1.00	-
Water deionised	73.50	76.50
Campo Enzymes Coupled Ascorbic acid Liquid	1.00	1.00
Citric acid	0.30	0.30
Songyi Gel 100 %	2.00	2.00
GE132 Organic Germanium sesquioxide	0.10	0.10
Snow White Coral Algae Extract	0.10	0.10
Perfume and Plantservative WS (Preservatives)	qs	qs

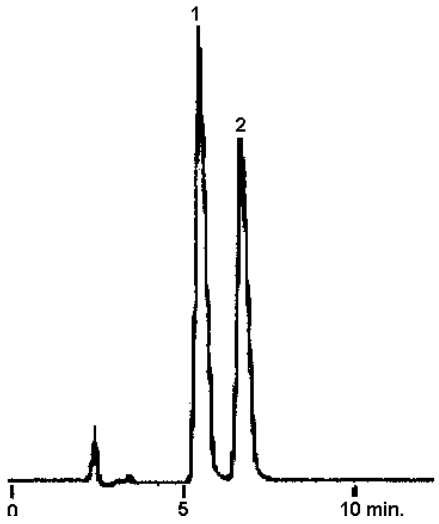
Method of manufacture

Add water to oil phase at 65/70^{o}C under stirrer. Allow to cool with stirring. At 40-45 ^{o}C add, in order, GE132 Organic Germanium sesquioxide-agitated until totally dissolved, Snow White Coral Algae, Enzymes Coupled Ascorbic Acid Liquid, Songyi Gel 100% and Citric Acid, Perfume at 40 ^{o}C. Adjust pH, when necessary, to 4.0-4.5 with Citric Acid.

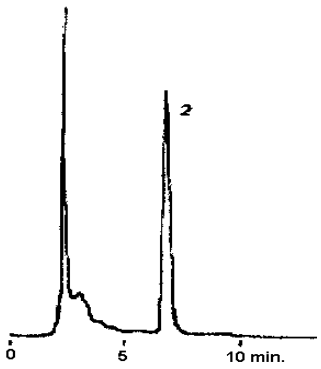
For the analysis of **Ascorbic acid**, amino or silica based columns are usually used. Analysis using amino columns is particularly wide spread because isoascorbic acid as well as ascorbic acid can be analyzed together. However, conventional amino columns are silica-based, bonded with aminopropyl groups or polyamine and are not chemically stable. Therefore, they have certain disadvantage such as a decrease in retention and short column life.

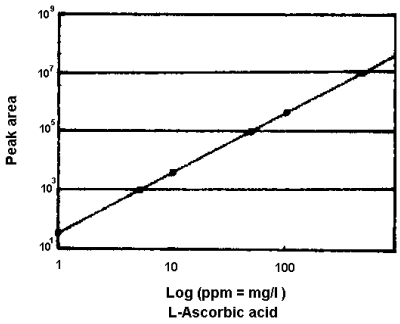
Since Asahipak NH2P–50 is polymer-based column, it does not suffer from such disadvantages. Moreover, since NH2P-50 column can be washed with alkaline solvents, the column life can be greatly extended. Asahipak NH2P-50 is used for analysis of **Enzymes Coupled Ascorbic Acid Liquid**

Sample: 20μl
Dissolved in 5% metaphosphoric acid
1. D-Isoascorbic acid 0.05 ppm
2. L- Ascorbic acid 0.05 ppm



Sample: 20μl
Enzymes Coupled
Ascorbic Acid Liquid





Sample:
Metaphosphoric Acid
1. D-Isoascorbic acid 2. L-Ascorbic Acid

Sample:
Enzymes Coupled Ascorbic Acid Liquid

Column : Shodex Asahipak NH2P-50 4E
Eluent : 60mM Phosphoric acid (pH 2.0) / CH_3CN = 20/80
Flow rate : 1.0 ml/min
Detector : Shodex UV (254 nm)
Column temp. : 40°C

"(SAFETY DATA SHEET – compliant to GHS)" CONFIRMS TO EC DIRECTIVE 91/155/EEC, EC REGULATION NO#1272/2008, AMENDED EC REGULATION NO#790/2009 and Complies to The EU Cosmetic Products Regulation (Regulation (EC) No 1223/2009) effective on July 2013., and to EU Commission Regulation No.358/2014/9 of 9th April 2014 amending Annexes II and V, to EU Regulation No No.1223/2009 of The European Parliament and of The Council on Cosmetic products, (Effective Date 31st October 2014) AND to US DEPT.OF LABOR-Occupational Safety & Health Admin directives and compliant to Globally Harmonized System of Classification and Labeling of Chemicals (hereinafter referred to as "the GHS")., and Complies and Confirms to the Requirements of State of California Proposition 65.

A Quality Management System, compliant to the International Standard ISO 9001, was used to manufacture and test this material.

http://www.osha.gov/dsg/hazcom/ghs.html
http://www.unece.org/trans/danger/publi/ghs/ghs_welcome_e.html
http://www.hc-sc.gc.ca/ahc-asc/intactiv/ghs-sgh/index-eng.php

	DATE OF ISSUE	Sept. 19 1997- Rev'wer- Dr Fergus Jes .G.Velasquez Bsc. Med Tech, MD
	DATE OF LATEST REVISION	**Mr Jimmy Kee, 30th June 2003** **Mr Teo SH 5th Jan 2004** **Balasubramaniam M,PhD 21st August 2007** **Mr Joshua Teo, 21 Jan 2011** **February 5th 2013 – Reviewer –** **Dr Balasubramaniam M, PhD** **23rd March 2014 - Joshua Teo Balasubramaniam M PhD & Oksana Nemchenko MD** **12th February 2015 - Joshua Teo BSc. Chem, Balasubramaniam M PhD & Oksana Nemchenko MD** **15th May 2016 - Joshua Teo BSc. Chem, Balasubramaniam M PhD & Oksana Nemchenko MD**
1	PRODUCT AND COMPANY IDENTIFICATION	
	COMMERCIAL NAME:	**CAMPO ENZYMES COUPLED ASCORBIC ACID LIQUID**
	OTHER NAMES	ENZYMES COUPLED ASCORBIC ACID LIQUID
	INCI NAME:	Ascorbic Acid (L-)
	Chinese Translation	抗坏血酸（维生素 C）
	INTERNATIONAL CHEMICAL IDENTIFICATION ***(EC REGULATION NO#1272/2008 AMENDED NO#790/2009)and Compliant to the GHS***:	**ASCORBIC ACID (L-)**
	MANUFACTURER : (cGMP MFG. FACILITIES) :	**CAMPO RESEARCH PTE LTD** **Hudson Industrial Bldg., #05-02,** **14, New Industrial Road, Singapore 536200**.
	EMERGENCY TELEPHONE NUMBERS:	(65)-63833631/(65)-63228503 (Singapore)
2	HAZARDS INDENTIFICATION	
	NOT CLASSIFIED AS DANGEROUS ACCORDING TO DIRECTIVE 67/548/EEC OR	DIVISION 1.6; NON-HAZARDOUS NO HAZARD STATEMENT

ITS AMENDMENTS.	
HAZARD CLASS and CATEGORY CODE(s)	PICTOGRAM : NONE
HAZARD STATEMENT CODE(s) ***(EC REGULATION NO#1272/2008 AMENDED NO#790/2009)*** **and compliant to the GHS**	No GHS Pictogram (Totally Non-Hazardous) Division 1.6; NO HAZARD STATEMENT
GHS CLASSIFICATION : This material is Non-hazardous according To UN-GHS Criteria.	PICTOGRAM : NONE No GHS Pictogram (Totally Non-Hazardous) Division 1.6: No Hazard Statement.
GHS LABEL ELEMENTS:	No GHS Pictogram (Totally Non-Hazardous) Division 1.6: No Hazard Statement.
3 COMPOSITION / INFORMATION ON INGREDIENTS	
ASCORBIC ACID-L, COUPLED WITH OXIDOREDUCTIVE ENZYMES	E.C. 1.8.5.1 Glutathione dehydrogenase; E.C. 1.11.1.11 L- ascorbate peroxidase E.C. 1.6.5.4 Monodehydroascorbate reductase E.C. 1.10.3.3 L-ascorbate oxidase **L-ascorbic acid**
CTFA Monograph ID:	213 – Ascorbic Acid
CAS#	50-81-7 – (L-) / 62624-30-0 – Ascorbic Acid
CAS# EU	50-81-7 / 62624-30-0 (EU) – Ascorbic Acid
CAS NO# (CAS Name) ***(EC REGULATION NO#1272/2008 AMENDED NO#790/2009)and compliant to the GHS***	**50-81-7 / 62624-30-0 – Ascorbic Acid**
EINECS Name and Number	200-066-2(1) / 263-644-3 (1) – Ascorbic Acid
EINECS# EU	200-066-2 / 263-644-3 (EU) – Ascorbic Acid
EINECS# (EINECS Name) ***(EC REGULATION NO#1272/2008 AMENDED NO#790/2009) and compliant to the GHS***	**200-066-2 / 263-644-3 – Ascorbic Acid**
EINECS Name and Number	Ascorbic Acid
EINECS# EU	http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=74328
European Commission–Health & Consumer Cosmetics–Cosing	Ascorbic Acid – 200-066-2 / 263-644-3 (EU)
RISK PHRASES	None
SAFETY PHRASES 25-26	Not Mandatory
GHS CLASSIFICATION : This material is Non-hazardous according To UN-GHS Criteria.	**PICTOGRAM : NONE**
GHS LABEL ELEMENTS:	**No GHS Pictogram (Totally Non-Hazardous) Division 1.6: No Hazard Statement.**
4 FIRST AID MEASURES	
EYE CONTACT:	Flush with water or standard eye wash solution for 20 to 30 minutes. Immediately transport the victim after flushing eyes to a hospital even if no symptoms (such as redness or irritation) develop
ORAL INGESTATION:	Do not induce vomiting. If victim is conscious and not convulsing, give 1 or 2 glasses of water to dilute the chemical. If unconscious and convulsing do not give by mouth, ensure airway is open and lay victim on his side with the head lower

		than the body.
	SKIN CONTACT:	Immediately flood affected skin with water then wash thoroughly with soap and water.
5	FIRE FIGHTING MEASURERS	
	COMBUSTIBLE BUT PRESENTS NO SPECIAL FIRE HAZARD.	
	EXTINGUISHING MEDIA:	CO2, dry foam or dry chemical or halon extinguisher.
	PROTECTIVE EQUIPMENTS FOR FIGHTERS:	Standard Equipments.
6	ACCIDENTAL RELEASE MEASURES	
	DAMPEN THE SOLID SPILL MATERIAL WITH WATER, THEN TRANSFER TO A SUITABLE CONTAINER. USE ABSORBENT PAPER DAMPENED WITH WATER TO PICK UP ANY REMAINING MATERIAL.	SEAL CONTAMINATED CLOTHING AND THE ABSORBENT PAPER IN A VAPOR-TIGHT PLASTIC BAG FOR EVENTUAL DISPOSAL.
7	HANDLING AND STORAGE	
	PROTECT FROM EXPOSURE TO LIGHT. KEEP THE CONTAINER TIGHTLY CLOSED UNDER INERT ATMOSPHERE AND STORE UNDER COOL TEMPERATURE.	
8	EXPOSURE AND PERSONAL PROTECTION	
	IN ACCORDANCE WITH GOOD INDUSTRIAL PRACTICE AND HANDLING USING STANDARD EYE PROTECTION.	
9	PHYSICAL AND CHEMICAL PROPERTIES	
	PHYSICAL FORM:	Liquid
	COLOUR:	Varies from light almost Colorless to Dark Brown
	ODOUR:	Odorless
	BOILING POINT:	100°C
	MELTING POINT:	-
	VISCOSITY: (mPa.s)	N/A
	FLASH POINT:	N/A
	FLAMMABILITY SOLID/GAS:	-
	AUTO FLAMMABILITY:	-
	SPECIFIC REFRACTIVE:	1.330 - 1.450
	EXPLOSIVE PROPERTIES:	N/A
	pH: (100% Concentrate)	1.00 – 3.50
	OXIDIZING PROPERTIES:	N/A
	VAPOUR PRESSURE:	N/A
	DENSITY:	1.050 - 1.150
	WATER SOLUBILITY:	Soluble
	OTHER SOLUBILITY:	DMSO; 95% ethanol ; methanol; acetone
	BULK DENSITY:	-
	PARTITION COEFFICIENT: (OCTANOL/WATER)	-
	EXPLOSIVE LIMITS:	-
10	STABILITY AND REACTIVITY	
	Sensitive to prolonged exposure to air and light. Also sensitive to moisture; Aqueous solution oxidized by air reaction is accelerated by alkalies, oxygen concentration and is catalyzed by metal ions.	Optimum pH is 5.4. UV spectrophotometric stability screening indicates that solutions of this chemical in 95% ethanol are stable for less than 2 hrs. In DMSO or acetone should be stable for 24 hrs under normal lab conditions.
11	TOXICOLOGICAL DATA	Animal Tests Last Done 1992, as requirements of the then **EC DIRECTIVE 91/155/EEC**
	IVN	TDLo =2300 mg/kg (Body Wt.) man TDLo =900 mg/kg (Body Wt.) wmn
	DERMAL:	Expected To Be Essentially Non Toxic.
	INHALATION:	Leave the contaminated area; take deep breaths of fresh air.
	SPECIFIC CONCENTRATION LIMITS M-FACTORS ***(EC REGULATION NO#1272/2008***	TDLo =2300 mg/kg (Body Wt.) man TDLo =900 mg/kg (Body Wt.) wmn

	AMENDED NO#790/2009) **compliant to the GHS.**	
	TOXIC EFFECTS:	
	Symptoms of exposure may include irritation of the skin, eyes and respiratory tract. Ingestion of large amounts may cause gastrointestinal distress and diarrhea.	*Summarized toxicological data as shown here are formation bounded under Non-Disclosure Agreement with various clients as when these Toxicological Data were established or their exclusive uses.*
12	ECOLOGICAL INFORMATION	
	BIODEGRATION:	Expected To Be Ultimately Biodegradable.
	FISH TOXICITY:	No Data
	BACTERIAL & VIRAL TOXICITY:	No data
	WGK CLASS:	WGK (Self Classification)
13	DISPOSAL CONDITIONS	
	DISPOSE OFF ACCORDING TO A RECOGNISED METHOD OF CHEMICAL WASTE DISPOSAL.	
14	TRANSPORT INFORMATION	
	UN NUMBER# :	N/A
	UN NAME:	Not Assigned
	IMDG CODE/CLASS:	Not Hazardous
	IMDG CODE PAGE NO.	N/A
	ICAO/IATA AIR CLASS:	Non-Hazardous
	ICAO/IATA AIR CLASS PACKING GROUP:	N/A
	RID/ADR CLASS:	Non-Hazardous
	ADNR CLASS:	Non-Hazardous
	(EC REGULATION NO#1272/2008 AMENDED NO#790/2009) and compliant to the GHS.	
	PICTOGRAM SIGNAL WORD CODE(s):	No GHS Pictograms (Totally Non-Hazardous)
	HAZARD STATEMENT CODE(s):	Division 1.6; No Hazard Statement
	SUPPLEMENTARY HAZARD STATEMENT CODE(s):	Similar Division 1.6; No Hazard Statement
15	REGULATORY INFORMATION	
	OCCUPATIONAL EXPOSURE LIMITS:	N/A
	U.S. State of California Proposition 65 INGREDIENTS Presence	**None (Exempted from CA Prop 65 Register)**
	EU Commission Regulation No.358/2014/9 of 9th April 2014 amending Annexes II and V, to EU Regulation No No.1223/2009 of The European Parliament and of The Council on Cosmetic products	**"Contains No Parabens and nor contains any Branched Chain Parabens".(**EU Regulation No.358/2014/9 of 9th April 2014)
16	OTHER INFORMATION	
	Occurs widely in plants. Has a wide variety of usage: in nutrition, color fixing, flavorings and preservation of meats and other foods. Used as reducing agent in analytical chemistry, as an antimicrobial and antioxidant in foodstuffs, in the treatment of Vitamin C deficiency, and in veterinary medicine to treat vitamin C deficiency in primates, guinea pigs and fish.	It is a dietary supplement and chemical preservative. It cures scurvy and increases resistance to infection. It acts as an oxidation-reduction catalyst in the cell.
	This format and information is compiled by Kampoyaki Novel Natural Product Chemistry/ Novel Drug Discovery cGMP Labs Kobe, Japan; for Campo Research, Kyoto and Singapore.	***Please take note that all specifications are liable to changes without prior notice.**

CAMPO ENZYMES COUPLED ASCORBIC ACID LIQUID ©. **© US. Library-of Congress 1989-2017©**

ASCORBIC ACID

Metabolism, like other aspects of life, involves tradeoffs. Oxidant by-products of normal metabolism cause extensive damage to DNA, protein, and lipid. This damage (the same as that produced by radiation) could possibly be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, immune-system decline, brain dysfunction, and cataracts.

Oxygen is a Yanus-faced molecule, it conditions life but it also exerts toxic effects. Superoxide and hydroxyl radicals together hydrogen peroxide and singlet oxygen are believed to be responsible for oxygen toxicity. It is supposed that free radical mechanism is participated in inflammatory diseases, myocardial infarction, carcinogenesis, in effect of ionizing radiation or in aging.

Protection is thought to be available in the form of endogenous compounds that react with and thereby "scavenge" these reactive free radicals. Because many free radicals are reactive forms of oxygen, an effective scavenger is often referred to as an antioxidant. To be an effective physiologically, a substance must have certain chemical and biological properties:

1. it must be present in adequate amounts in the body
2. it must react with a reactive free radical species
3. it must be suitable for compartmentation
4. it must be readily available
5. it might be suitable for regeneration
6. it must be conserved by the kidneys
7. and it must have tolerable toxicity.

There is an enormous amount of literature on vitamin C intake and health in animals, cell cultures, and humans. Beyond its function in collagen formation, ascorbic acid is known to increase absorption on inorganic iron, to have essential roles in the metabolism of folic acid and some of amino acids and hormones, and to act as antioxidant. In recent years, research has increasingly focused on this later function, stimulated by suggestions that "oxidative stress" may be a causal factor in the etiology of such diverse and important disorders of aging as cancer, cardiovascular disease, and cataract formation. It has been appreciated that ascorbic acid has important functions in many cellular reactions and processes in addition to its role in collagen synthesis.The few such reaction that are understood at the molecular level make it apparent that ascorbic acid does not directly participate in enzyme - catalyzed conversion of substrate to product. Instead, the vitamin regenerates prosthetic metal ions in these enzymes in their required reduced forms. This is in agreement with other antioxidant functions of vitamin C, e.g, scavenging of free radicals. Ascorbate and other antioxidant nutrients are presumed to play a pivotal role is twofold: the already-oxidized groups in prosthetic centers of enzymes are reduced and the oxidants and free radicals are removed…"

Ascorbic acid (vitamin C) as well as the carotenoids react with free radicals, notably peroxyl radicals, and with singlet molecular oxygen (102), this being the basis of their function as antioxidants. L-Ascorbate is present in aqueous

compartments (e.g. cytosol, plasma, and other body fluids) and can reduce the tocopheroxyl radical ; it also has a number of metabolically important cofactor functions in enzyme reactions, notably hydroxylations. Upon oxidation, these micronutrients need to be regenerated in the biological setting, hence the need for further coupling to non radical reducing systems such as glutathione/glutathione disulfide, dihydrolipoate/lipoate, or NADPH/NADP+ and NADH/NAD+. Antioxidant functions are associated with lowering DNA damage, malignant transformation, and other parameters of cell damage in vitro as well as epidemiologically with lowered incidence of certain types of cancer and degenerative diseases, such as ischemic heart disease and cataract. They are of importance in the process of aging. Reactive oxygen species occur in tissues and cells and can damage DNA, proteins, carbohydrates, and lipids. These potentially deleterious reactions are controlled in part by antioxidants that eliminate pro oxidants and scavenge free radicals. Their ability was antioxidants to quench radicals and 102 may explain some anticancer properties of the vitamin C activity, but other functions may explain some anticancer properties of the vitamin C activity, but their functions may play a role as well.

Dehydroascorbic acid is generated in plants and animals cells by oxidation of ascorbic acid. The reaction is believed to occur by the one-electron oxidation of ascorbic acid to semihydroascorbate radical followed by dispropotionation to dehydroascorbic acid and ascorbic acid.

Semihydrascorbic acid , may recycle to ascorbic acid catalyzed by membrane-bound NADH-semihydroascorbate reductase. However, disproptionation of the free radical occurs at a rapid rate,10 (5) M-1s-1, accounting for measurable cellular levels of dehydrascorbate. Dehydrascorbate reductase, studied earlier and more extensively in plants, is now recognized as the intrinsic activity of thioltransferases (glutaredoxins) and proteins disulfide isomerase in animal cells. These enzymes catalyze the glutathione-dependent two electron regeneration of ascorbic acid. The importance of the latter route of ascorbic acid renewal was seen in studies of GSH-deficient rodents (Meister, A. (1992) Biochem oxidation of nascent protein dithiols to disulfides catalyzed in the endoplas . Pharmacol .44, 1905-1915). GSH deficiency in newborn animals resulted in decreased tissue ascorbic acid and increased dehydrascorbate-to-ascorbate ratios. Administration of ascorbic acid daily to GSH-deficient animals decreased animal mortality and cell damage from oxygen stress. A cellular role is proposed for dehydroascorbate in the mic recticulum compartment by protein disulfide isomerase.

ENTRY	**EC 1.10.3.3**
NAME	L-Ascorbate oxidase
	Ascorbase
CLASS	Oxidoreductases
	Acting on diphenols and related substances as donors
	With oxygen as acceptor
SYSNAME	L-Ascorbate:oxygen oxidoreductase
REACTION	2 L-Ascorbate + O2 = 2 Dehydroascorbate + 2 H2O
SUBSTRATE	L-Ascorbate
	O2
PRODUCT	Dehydroascorbate
	H2O
COFACTOR	Copper
COMMENT	A multicopper protein.

ENTRY	**EC 1.6.5.4**
NAME	Monodehydroascorbate reductase (NADH)
CLASS	Oxidoreductases
	Acting on NADH or NADPH
	With quinone or related compound as acceptor
SYSNAME	NADH:monodehydroascorbate oxidoreductase
REACTION	NADH + 2 Monodehydroascorbate = NAD+ + 2 Ascorbate
SUBSTRATE	NADH
	Monodehydroascorbate
PRODUCT	NAD+
	Ascorbate

ENTRY	**EC 1.11.1.11**
NAME	L-Ascorbate peroxidase
CLASS	Oxidoreductases
	Acting on a peroxide as acceptor
SYSNAME	L-Ascorbate:hydrogen-peroxide oxidoreductase
REACTION	L-Ascorbate + H2O2 = Dehydroascorbate + 2 H2O
SUBSTRATE	L-Ascorbate
	H2O2
PRODUCT	Dehydroascorbate
	H2O

ENTRY	**EC 1.8.5.1**
NAME	Glutathione dehydrogenase (ascorbate)
CLASS	Oxidoreductases
	Acting on a sulfur group of donors
	With a quinone or similar compound as acceptor
SYSNAME	Glutathione:dehydroascorbate oxidoreductase
REACTION	2 Glutathione + Dehydroascorbate = Oxidized glutathione + Ascorbate
SUBSTRATE	Glutathione
	Dehydroascorbate
PRODUCT	Oxidized glutathione
	Ascorbate

ASCORBIC ACID

TECHNICAL SPECIFICATION:

PRODUCT NAME: Campo Enzymes Coupled Ascorbic Acid Powder

PRODUCT NUMBER: 97.57784

BASE CHEMICAL NAME: Ascorbic acid, L- (CAS# 50-81-7)
Coupled enzymes E.C.#
E.C. 1.10.3.3 (L-Ascorbate oxidase)
E.C. 1.6.5.4 (NADH)
E.C. 1.11.1.11 (L-ascorbate peroxidase)
E.C. 1.8.5.1 (Glutathione dehydrogenase)

CHEMICAL FORMULA: $C_6H_8O_6$

APPEARANCE: White crystals

ODOUR: Odourless

DENSITY: 1.65

MELTING POINT: 190 - 192°C (decomposes)

SOLUBILITIES: Water (100 mg/ml at 23°C)
DMSO (100 mg/ml at 23°C)
95% Ethanol (< 1mg/ml at 23°C)
Methanol (1 in 10)
Acetone (<1 mg/ml at 23°C)

OTHER PHYSICAL DATA: Pleasant, sharp acidic taste

COMMENTS: This may be sensitive to prolonged exposure to air and light. It is also sensitive to moisture. Aqueous solutions are oxidized by air. This reaction is accelerated by alkalies, iron and copper. The oxidation rate is dependent on the pH and on oxygen concentration and is catalyzed by metal ions. The optimum pH is 5.4. This is also subject to degradation under anaerobic conditions.

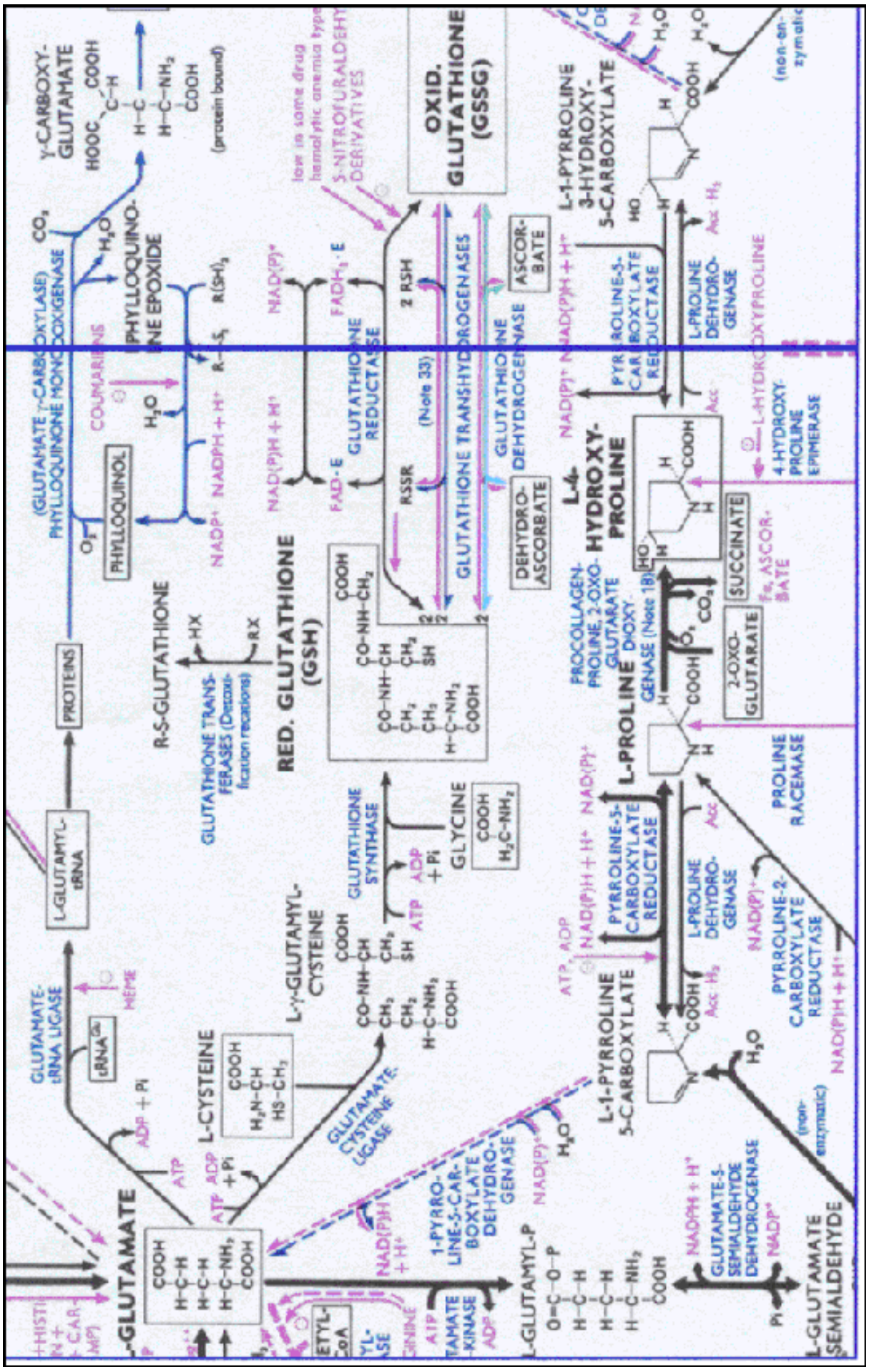
Sample Formulary:

Skin Whitening cream		
Ingredients	**321418-C1179 CPWD**	**2840-C1420 CPWD**
	%	**%**
Ceramide Oil and Vegetal Lanolin Alcohol	7.00	7.00
Cetearyl alcohol	3.00	3.00
Glyceryl stearate	10.00	-
Glyceryl stearate and PEG-100 Stearate	-	10.00
PEG-40-castor oil	2.00	-
DEA-Oleth-3-phosphate	1.00	-
Water deionised	74.30	77.30
Campo Enzymes coupled Ascorbic acid Powder	0.20	0.20
Citric acid	0.30	0.30
Songyi Gel 100 %	2.00	2.00
Campo Pearl Extract PWS	0.10	0.10
Snow White Coral Algae Extract	0.10	0.10
Perfume and Plantservative WS (Preservatives)	qs	qs

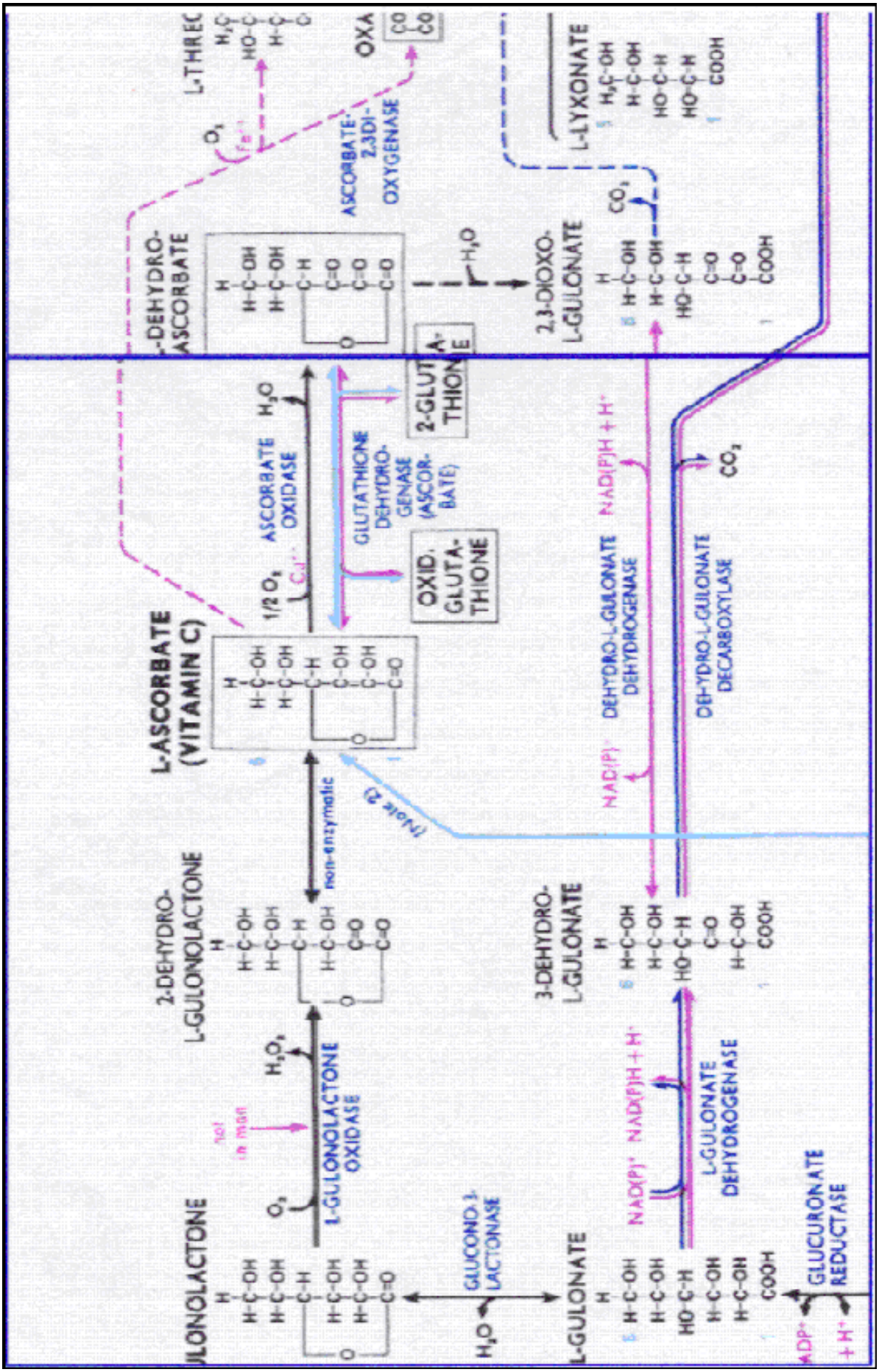
Method of manufacture

Add water to oil phase at 65/70^{o}C under stirrer. Allow to cool with stirring. At 40-45 ^{o}C add, in order, Campo Pearl Extract PWS-agitated until totally dissolved, Snow White Coral Algae, Enzymes Coupled Ascorbic Acid Powder, Songyi Gel 100% and citric acid, Perfume at 40 ^{o}C. Adjust pH, when necessary, to 4.0-4.5 with citric acid.

Biocampo Novel Drug Discovery Database
For Biochemical Pathways Map No. B1



BioCampo Novel Drug Discovery Database
for Biochemical Pathways Map No. B2



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ENZYME: EC 1.10.3.3

Official Name:
L-ASCORBATE OXIDASE.

Alternative Name(s):
ASCORBASE.

Reaction catalysed:
2 L-Ascorbate + O(2) <=> 2 DEHYDROASCORBATE + 2 H(2)O

Cofactor(s): COPPER.

Cross-Reference(s):
- Biochemical Pathways; map number(s): B2
- PROSITE: PDOC00076.
- EMP/PUMA: 1.10.3.3
- WIT: 1.10.3.3
- KYOTO UNIVERSITY LIGAND CHEMICAL DATABASE: 1.10.3.3
- SWISS-PROT:
  Q00624, ASO_BRANA; P24792, ASO_CUCMA; P37064, ASO_CUCPM;
  P14133, ASO_CUCSA; Q40588, ASO_TOBAC;

ENTRY **EC 1.10.3.3**

NAME L-Ascorbate oxidase
Ascorbase

CLASS Oxidoreductases
Acting on diphenols and related substances as donors
With oxygen as acceptor

SYSNAME L-Ascorbate:oxygen oxidoreductase

REACTION 2 L-Ascorbate + O_2 = 2 Dehydroascorbate + 2 H_2O

SUBSTRATE L-Ascorbate
O_2

PRODUCT Dehydroascorbate
H_2O

COFACTOR Copper

COMMENT A multicopper protein.

Enzyme Structures Database

E.C.1.-.-.-Oxidoreductases.

E.C.1.1O.-.-Acting on diphenols and related substances as donors.

E.C.1.10.3.-With oxygen as acceptor.

E.C.1.10.3.3 L-Ascorbate oxidase.

Reaction: *2 L-Ascorbate + O(2) = 2 dehydroascorbate + 2 H(2)O.*
Other name(s): *Ascorbase.*
Cofactor(s): *Copper.*

There are **4** PDB entries in enzyme class E.C.1.10.3.3

laoz (CATH classification *Chain A* **2.60.40.170** , **2.70.80.10** , *Chain B* **2.60.40.170** , **2.70.80.10**)
Structure: *Ascorbate oxidase*
Source: *Zucchini (cucurbita pepo medullosa)*

laso (CATH classification *Chain A* **2.60.40.170** , **2.70.80.10** , *Chain B* **2.60.40.170** , **2.70.80.10**)
Structure: *Ascorbate oxidase (reduced form)*
Source: *Zucchini (cucurbita pepo medullosa)*

lasp (CATH classification *Chain A* **2.60.40.170** , **2.70.80.10** , *Chain B* **2.60.40.170** , **2.70.80.10**)
Structure: *Ascorbate oxidase (peroxide form)*
Source: *Zucchini (cucurbita pepo medullosa)*

lasq (CATH classification *Chain A* **2.60.40.170** , **2.70.80.10** , *Chain B* **2.60.40.170** , **2.70.80.10**)
Structure: *Ascorbate oxidase (azide form)*
Source: *Zucchini (cucurbita pepo medullosa)*

E.C. number	1.10.3.3	
Systematic name	L-Ascorbate:oxygen oxidoreductase	
Recommended name	L-Ascorbate oxidase	
Other names	Oxidase, ascorbate; Ascorbic acid oxidase; Ascorbate oxidase; Ascorbase; Ascorbic oxidase; Ascorbate dehydrogenase; L-Ascorbic acid oxidase; AAO ; L-Ascorbate:O2 oxidoreductase ; AA oxidase	
CAS registration no.	9029-44-1	
Reaction catalysed	2 L-Ascorbate + O_2 = 2-dehydroascorbate + H_2O	
Reaction type	Redox reaction	
Substrate spectrum	**1.**	L-Ascorbate + O2
	2.	D-Glucoascorbic acid + O2
	3.	D-Isoascorbic acid + O2
	4.	More (substrate specificity: overview highly specific for ascorbic acid (and a few of its analogs and O2) , anionic form of the substrate is an important requirement of the enzyme specificity , mechanism: Cu2+ is reduced to Cu+, which is then reoxidized by oxygen ,double displacement mechanism (enzymatic memory) , low reaction rate with bilirubin, at pH 5.7: oxidation of leuco 2,6-dichloroindophenol to the blue quinoid dye , oxidation of 2,6- and 2,5-dichlorohydroquinone and hydroxyquinone at a rate about 1/12 of ascorbic acid , no oxidation of hydroquinone , not appreciably oxidized: p-phenylenediamine , Na2S2O3 , glutathione , cysteine, ascorbate oxidase activity of caeruloplasmin .
Products	**1.**	L-Dehydroascorbate + H_2O
	2.	Glucodehydroascorbate + H_2O
	3.	2-Dehydroisoascorbate + H_2O
	4.	?
Natural substrates	L-Ascorbate + O2 (possibly a kind of pathogenesis-related protein ;	
Turnover number		
Specific activity		
Km value	0.100-0.350 (L-ascorbate, free enzyme); 0.200 (L-ascorbate, spectrophotometric method, Cucurbita pepo condensa); 0.98 (L-ascorbate, Warburg method, Cucurbita pepo condensa) ; 0.36 (L-ascorbate, spectrophotometric method, Cucurbita pepo medullosa) ; 2.21 (L-ascorbate, Warburg method) ; 0.181 (L-ascorbate, spectrophotometric method, Cucumis sativus) ; 1.125 (L-ascorbate, Warburg method, Cucumis sativus) ; More (Km of the native enzyme and various deglycosylated forms , Km of ascorbate and O2 is insensitive to pH in the range 5-8.5	
pH optimum	5.5-7.0 ; 5.6 (free and immobilized enzyme ; 6.0	
pH range	4.5-8.3 (at pH 4.5 and 8.3: about 50% of activity maximum) ; 5-9.0 (5.0: about 60% of activity maximum (free enzyme), about 80% of activity maximum (immobilized enzyme), 9.0: less than 10% of activity maximum (free enzyme), about 45% of activity maximum (immobilized enzyme)	

Temperature optimum	
Temperature range	
Cofactors/prosthetic groups	H2O2 (stimulation at low concentration, 0.056 mM) ; o-Phenanthroline (stimulation) ; Bipyridyl (stimulation) ; Ethanol (stimulation) p-Chloromercuribenzoate (stimulation) ; Iodoacetate (stimulation)
Metal ions/salts	Copper (a multicopper protein, 6 atoms of copper per enzyme molecule , contains 8 atoms of copper per enzyme molecule (of MW 132000, 140000 , contains 10-12 atoms of copper per enzyme molecule of MW 140000, mononuclear blue copper in domain 3 and trinuclear copper between domain 1 and 3 , measurement of intramolecular electron transfer between type I and type III copper centers in the multi-copper enzyme, contains two type 1, two type 2 and four type 3 copper ions , electronic structure of blue copper sites , coordination environment of type 2 copper, principal active site comprised of one type I, one type II and a pair of type III coppers ; More (enzyme from Myrothecium verrucaria: no support of a metal in the enzyme)
Inhibitors	Hg2+ ; Ni2+ (some authors report inhibition, others not) Zn2+ (some authors report inhibition, others not) Cu2+ Cyanide (very slight) Diethyldithiocarbamate (not KNO3 Nitrofurantoin (slight); H2S ; Ethyl xanthate; 8-Hydroxyquinoline; Pyridine-KNCS; Thiourea; Salicylaldoxime; Azide (mixed-type inhibition above pH 6, competitive inhibition at pH 5.6, weak); Leucocyanidol; 3,4-Dichlorophenylserine; Tetraethylthiuramidisulfide; Cupferron; Carotenes; SO2; Deoxycorticosterone; Anthocyanin pigments; H2O2 (inhibition at 5.6 mM, stimulation at 0.56 mM; alpha-Tocopherol ; Propylgallate; Nordihydroguaiaretic acid; Thiamine ; Auxin analogs ; Organic mercurials; Iodoacetate ; p-Substituted mercuribenzoate (some authors report inhibition, others not); F- ; Citrate (univalent anion); Piperazine N,N'-bis(2-ethanesulfonic acid) (anions) ; NaNO2 ; Lauryl sulfate; Fenton's reagent (Fe2+ + H2O2 + 2 H+) ; Urea (effect on various molecular forms); More (natural inhibitors: cabbage extract, tomato extract, strawberry juice, extract of lemons, oranges, parsley, hips, leeks, Myrothecium verrucaria, reaction inactivation: progressive loss of activity during oxidation of ascorbic acid)

Organism (original)	Cucurbita pepo condensa (yellow summer crookneck, yellow summer squash, 5 isoenzymes, 5 molecular forms: monomer, tetramer, octamer, dodecamer, polymer; Cucumis sativus (cucumber, 3 molecular forms: monomer, dimer, tetramer; Cucurbita pepo medullosa; Myrothecium verrucaria; Brassica oleracea (cabbage); Sinapis alba (mustard) ; Cucurbita spp. (Ebisu Nankin); Cucurbita moschata; Cucurbita maxima; More (ascorbate oxidase activity of human caeruloplasmin)
Source	Medium (of cultured cells of Cucumis sativus, Cucurbita spp. Seeds ; Leaves; Peel; Fruit; Cotyledons; Cultured cells (of Cucumis sativus)
Localization	Soluble; Cell-wall; Cytoplasm; Extracellulr (cultured cells)
Purification	Cucumis sativus; Cucurbita pepo medullosa; Myrothecium verrucaria
Crystallization	(Cucurbita pepo medullosa, X-ray crystal structure)
Molecular Weight	132000 (Cucumis sativus, sedimentation equilibrium); 140000 (Cucurbita pepo medullosa, sedimentation and difussion studies); 150000 (Cucurbita maxima, gel filtration)
Subunits	Monomer (1 * 30000, Cucumis sativus, SDS-PAGE, enzyme also exists as dimer and tetramer, 1 * 35000, Cucurbita pepo, SDS-PAGE, enzyme also exists as tetramer, octamer, dodecamer and polymer); Dimer (2 * 70000, MW 70000 subunit consists of 2 polypeptide chains: MW 30000 and 40000, Cucurbita pepo medullosa, 2 * 65000, MW 65000 subunit consists of 2 chains: A chain (38000) and B chain (28000), Cucurbita pepo medullosa, 2 * 30000, Cucumis sativus, SDS-PAGE, enzyme exists as monomer, dimer and tetramer); Tetramer (4 * 30000, Cucumis sativus, SDS-PAGE, enzyme exists as monomer, dimer and tetramer, 4 * 35000, Cucurbita pepo, SDS-PAGE, enzyme exists as monomer, tetramer, octamer, dodecamer and polymer); Octamer (8 * 35000, Cucurbita pepo, SDS-PAGE, enzyme exists as monomer, tetramer, octamer, dodecamer and polymer); Dodecamer (12 * 35000, Cucurbita pepo, SDS-PAGE, enzyme exists as monomer, tetramer, octamer, dodecamer and polymer); Polymer (x * 35000, Cucurbita pepo, MW between 670000 and 2000000, SDS-PAGE, enzyme exists as monomer, tetramer, octamer, dodecamer and polymer); More (quarternary structure)
Carbohydrate/ Lipid	Glycoprotein (enzyme may be a protein-copper carbohydrate complex, 2.4% carbohydrate , deglycosylation)
Cross-refs to NBRF/PIR	
Cloned	
Cross-refs to Brookhaven	
pH stability	4 (irreversible loss of activity below)
Temperature stability	0-40 (30 min, stable); 12 (stable for at least 30 days); 15 (immobilized enzyme retains full activity for 3 months at pH 5-7, free enzyme: pH 5-7, 40-70% loss of activity within one day); 40-50 (conversion of octamer and heavier forms to a dimer); 60 (20 min, 90% loss of activity (free enzyme), 10% loss of activity (immobilized enzyme));

	80 (10 min, complete loss of activity); 100 (1 min, all forms of enzyme inactivated); More (role of copper in heat stability, different molecular forms vary in resistance to heat inactivation: tetramer of squashes and dimer of cucumber being most resistant)
Organic solvent stability	
Oxidation stability	
General stability	Role of copper in stability; Gelatin protects against inactivation; Catalase protects against inactivation; Peroxidase protects against inactivation; Methemoglobin protects against inactivation; Immobilization within 6% Ca-alginate gel beads improves stability; 20-25% retention of activity after immobilization, at 12°C, stable for at least 30 days ; Stable to dialysis against EDTA or cyanide
Storage stability	4°C, concentrated solution, 3 months
Renaturated	-

ENZYME: EC 1.6.5.4

Official Name:
MONODEHYDROASCORBATE REDUCTASE (NADH).

Reaction catalysed:
NADH + 2 MONODEHYDROASCORBATE <=> NAD(+) + 2 ASCORBATE

Cross-Reference(s):
- EMP/PUMA: 1.6.5.4.
- WIT: 1.6.5.4.
- KYOTO UNIVERSITY LIGAND CHEMICAL DATABASE: 1.6.5.4.

ENTRY **EC 1.6.5.4**

NAME Monodehydroascorbate reductase (NADH)

CLASS Oxidoreductases
Acting on NADH or NADPH
With quinone or related compound as acceptor

SYSNAME NADH:monodehydroascorbate oxidoreductase

REACTION NADH + 2 Monodehydroascorbate = NAD+ + 2 Ascorbate

SUBSTRATE NADH
Monodehydroascorbate

PRODUCT NAD+
Ascorbate

E.C. number	1.6.5.4
Systematic name	NADH:monodehydroascorbate oxidoreductase
Recommended name	Monodehydroascorbate reductase (NADH)
Other names	NADH:semidehydroascorbic acid oxidoreductase; MDHA; Semidehydroascorbate reductase; AFR ; AFR-reductase; Ascorbic free radical reductase; Ascorbate free radical reductase; SOR; MDAsA reductase (NADPH); SDA reductase ; NADH:ascorbate radical oxidoreductase; NADH-semidehydroascorbate oxidoreductase; Ascorbate free-radical reductase; NADH:AFR oxidoreductase
CAS registration no.	9029-26-9
Reaction catalysed	NADH + 2 monodehydroascorbate = NAD+ + 2 ascorbate
Reaction type	Redox reaction
Substrate spectrum	**1.** NADH + monodehydroascorbate (other electron acceptors: 2,6-dichlorophenolindophenol , cytochrome b5, cytochrome c (+ ascorbate), monodehydroisoascorbate, D-isoascorbic acid, ferricyanide, 1,2-naphthoquinone-4-sulfonate , methylene blue (+ ascorbate), semidehydro-D(-)-ascorbic acid reduced more rapidly than semidehydro-L(+)-ascorbic acid, Fe3+ in complex form is reduced by NADH in presence of the enzyme)
Products	**1.** NAD+ + ascorbate
Natural substrates	NAD(P)H + monodehydroascorbate (enzyme sustains glyoxysomal NAD+ during beta-oxidation, the glyoxylate cycle and gluconeogenesis in the endosperm during germination, key enzyme for maintaining the ascorbic acid system in the reduced state, regeneration of ascorbate from monodehydroascorbate, physiologically NADPH is the electron donor , involvement in oxidation of NADH by lipid peroxide in mitochondria and microsomes, functions to provide cytoplasmic reducing equivalents to intramitochondrial cytochrome P-450)
Turnover number	More; 9000 (NADPH + monodehydroascorbate); 12000 (NADH + monodehydroascorbate)
Specific activity	39.5; 0.264; 61.0; 256
Km value	0.0012 (semidehydroascorbate); 0.12 (NADH); 0.210 (NADPH); 0.007 (NADH); 0.077 (NADH); 0.30 (NADPH); 0.05 (NADH); 0.005 (semidehydroascorbate); 0.0046 (NADH); 0.023 (NADPH); 0.0014 (monodehydroascorbate)
pH optimum	7.0 ; 7.0-7.2; 7.4 ; 7.5-8.5 ; 8

pH range	5.5-9.5 (pH 5.5: about 60% of activity maximum, pH 9.5: about 80% of activity maximum); 6.6-7.9 (at pH 6.6 and 7.9 about 50% of activity maximum)
Temperature optimum	39; 41
Temperature range	
Cofactors/prosthetic groups	NADH (activity threefold higher with NADPH than with NADH); NADPH (not active with NADPH, activity threefold higher with NADPH than with NADH; o-Phenanthroline (stimulation); Cytochrome b5 (contains cytochrome b5); Flavin (contains a flavin); FAD (contains 1 mol of FAD per mol of enzyme)
Metal ions/salts	Diphosphate (stimulation)
Inhibitors	Citrate (slight); Sodium diethyldithiocarbamate; Quinaldic acid; alpha,alpha'-Bipyridyl; 1-(2-Thenoyl)-3,3,3-trifluoroacetone; Cu2+; Zn2+ ; Tris-buffer (2-amino-2-(hydroxymethyl)-1,3-propanediol); Tes-buffer (2-([2-hydroxy-1,1-bis(hydroxymethyl)ethyl]aino)ethanesulfonic acid); Imidazole buffer; Phosphate buffer; Mn2+ ; p-Chloromercuribenzoate; 2-Iodoacetamide; N-Ethylmaleimide (inhibition partially reversed by thiol-containing compounds); Mersalyl (inhibition partially reversed by thiol-containing compounds, pyridine nucleotides protect); More (inhibition of membrane-bound enzyme by lectins, insulin inhibits enzyme in plasma membrane)
Organism (original)	Cuscuta reflexa (parasitic plant, activity is ten times lower than in etiolated and green plants); Neurospora crassa; Glycine max (soybean); Nicotiana tabacum (tobacco); Ricinus communis (castor bean); Rat ; Euglena gracilis Z; Solanum tuberosum (potato); Human; Pig; Jerusalem artichoke; Onion; Broad bean; Cauliflower ; Cucumber (Cucumis sativus); Pea ; Pterocladia sp.; Gigartina sp.; Hypnea sp.; Gracilaria sp.; Mouse
Source	Adrenal gland ; Heart ; Brain ; Lung; Spleen ; Polymorphonuclear leukocytes ; Kidney ; Cell ; Root nodules;

	Root ; Cultured cells ; Callus; Leaf ; Seedlings; Liver; Tubers ; Bud; Etiolated hook; Fibroblasts; Virus-transformed mouse fibroblasts; Fruit
Localization	Membrane (glyoxysomal membrane, plasma membrane , outer mitochondrial membrane; Mitochondria; Cytoplasm ; Cytosol; Microsomes ; More (localized in a hitherto not identified vesicle fraction)
Purification	Neurospora crassa; Solanum tuberosum; Euglena gracilis Z (partial); Cucumis sativus
Crystallization	-
Molecular Weight	42000 (Solanum tuberosum, gel filtration); 47000 (Cucumis sativus, gel filtration); 52000 (Euglena gracilis) ; 66000 (Neurospora crassa, gel filtration)
Subunits	Monomer (1 * 42000, Solanum tuberosum, 1 * 47000, Cucumis sativus, gel filtration)
Carbohydrate/ Lipid	-
Cross-refs to NBRF/PIR	
Cloned	-
Cross-refs to Brookhaven	
pH stability	
Temperature stability	43 (pH 6.0-7.4, stable); 50 (pH 6.0-7.4, unstable); 60 (1 min, 20% loss of activity, 14 min, complete inactivation)
Organic solvent stability	
Oxidation stability	
General stability	
Storage stability	4°C, Tricine-sodium hydroxide buffer, pH 8, 30 days, complete loss of activity, addition of 1-10 mM MgSO4: 62% loss of activity
Renaturated	-
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ENZYME: EC 1.11.1.11

Official Name:
L-ASCORBATE PEROXIDASE.

Reaction catalysed:
L-ASCORBATE + H(2)O(2) <=> DEHYDROASCORBATE + 2 H(2)O

Cross-Reference(s):
- PROSITE: PDOC00394 .
- EMP/PUMA: 1.11.1.11.
- WIT: 1.11.1.11.
- KYOTO UNIVERSITY LIGAND CHEMICAL DATABASE: 1.11.1.11.
- SWISS-PROT:
  Q05431, APX1_ARATH; P48534, APX1_PEA ;

ENTRY **EC 1.11.1.11**

NAME L-Ascorbate peroxidase

CLASS Oxidoreductases
Acting on a peroxide as acceptor

SYSNAME L-Ascorbate:hydrogen-peroxide oxidoreductase

REACTION L-Ascorbate + H_2O_2 = Dehydroascorbate + 2 H_2O

SUBSTRATE L-Ascorbate
H_2O_2

PRODUCT Dehydroascorbate
H_2O

E.C. number	1.11.1.11	
Systematic name	L-Ascorbate:hydrogen-peroxide oxidoreductase	
Recommended name	L-Ascorbate peroxidase	
Other names	L-Ascorbic acid peroxidase; L-Ascorbic acid-specific peroxidase; Peroxidase, ascorbate; Ascorbate peroxidase; Ascorbic acid peroxidase	
CAS registration no.		
Reaction catalysed	L-Ascorbate + H_2O_2 = dehydroascorbate + 2 H_2O	
Reaction type	Redox reaction; Peroxidation	
Substrate spectrum	**1.**	L-Ascorbate + H2O2
	2.	Pyrogallol + H2O2
	3.	Guaiacol + H2O2
	4.	o-Dianisidine + H2O2
	5.	D-Araboascorbic acid + H2O2
	6.	2,2'-Azino-di-[3-ethylbenzothiazoline-(6)-sulfonic acid]+ H2O2
	7.	Iodide + H2O2
	8.	Reductic acid + H2O2 (i.e. 2,3-dihydroxy-2-cyclopenten-1-one)
	9.	L-Ascorbic acid + tert-butylhydroperoxide
	10.	L-Ascorbic acid + cumene hydroperoxide
Products	**1.**	Dehydroascorbate + 2 H_2O
	2.	?
	3.	?
	4.	?
	5.	?
	6.	?
	7.	?
	8.	?
	9.	?
	10.	?
Natural substrates	L-Ascorbate + H2O2	
Turnover number		
Specific activity	254; 34.2;	
Km value	2.9 (ascorbate, enzyme form C); 6.5 (ascorbate, enzyme form B); 0.41 (L-ascorbate); 7.4 (guaiacol, enzyme form C); 0.056 (H2O2); 9.6 (pyrogallol); More (Hill plot)	
pH optimum	5.2 (enzyme form B); 6.2 (enzyme form C)	
pH range	5-8	
Temperature optimum	37	
Temperature range		
Cofactors/prosthetic groups	Heme	
Metal ions/salts	Fe (hemoprotein)	
Inhibitors	CN- ;	

	N3-; CO; C2H2; 2-Mercaptoethanol; Dithiothreitol; Reduced glutathione; Hg2+ ; Mn2+ ; Br- ; Zn2+ ; Al3+ ; Mg2+ ; Ca2+ ; Ni2+ ; Li+ ; I- ; F- ; EDTA
Organism (original)	Glycine max ; Vigna unguiculata; Vicia faba ; Vicia sativa ; Arachis hypogaea ; Lupinus alba (low activity); Medicago sativa ; Trifoleum subterraneum; Pisum sativum (pea); Alnus rubra ; Euglena gracilis; Wheat ; Spinacia oleracea (spinach)
Source	Root (nodules); Leaves ; Shoots
Localization	Chloroplast; Cytosol ; More (not in mitochondria, chloroplasts, microsomes)
Purification	Glycine max ; Pisum sativum; Euglena gracilis; Spinacia oleracea
Crystallization	-
Molecular Weight	47000 (Glycine max, gel filtration) ; 57000 (Pisum sativum, gel filtration); 76000 (Euglena gracilis, gel filtration) ; 30000 (Spinacia oleracea)
Subunits	Monomer (1 * 30000, Glycine max, SDS-PAGE, discrepancy to value from gel filtration probably due to enzyme conformation)
Carbohydrate/Lipid	-
Cross-refs to NBRF/PIR	
Cloned	-
Cross-refs to Brookhaven	
pH stability	
Temperature stability	4 (half-life 10 h) ; 40 (inactivated after 5 min); 52 (complete inactivation); 70 (5 min, complete inactivation)]
Organic solvent	

stability		
Oxidation stability	Not stable under aerobic conditions; Stabilization by ascorbate and sorbitol	
General stability		
Storage stability	-20°C or -80°C, crude extracts of root nodules, 50 mM potassium phosphate buffer, pH 7.0, several months	
Renaturated	-	
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ENZYME: EC 1.8.5.1

Official Name:
GLUTATHIONE DEHYDROGENASE (ASCORBATE).

Reaction catalysed:
2 GLUTATHIONE + DEHYDROASCORBATE <=> OXIDIZED GLUTATHIONE + ASCORBATE

Cross-Reference(s):
- Biochemical Pathways; map number(s): B2, H7, I7
- EMP/PUMA: 1.8.5.1.
- WIT: 1.8.5.1.
- KYOTO UNIVERSITY LIGAND CHEMICAL DATABASE: 1.8.5.1.

ENTRY **EC 1.8.5.1**

NAME Glutathione dehydrogenase (ascorbate)

CLASS Oxidoreductases
Acting on a sulfur group of donors
With a quinone or similar compound as acceptor

SYSNAME Glutathione:dehydroascorbate oxidoreductase

REACTION 2 Glutathione + Dehydroascorbate = Oxidized glutathione + Ascorbate

SUBSTRATE Glutathione
Dehydroascorbate

PRODUCT Oxidized glutathione
Ascorbate

E.C. number	1.8.5.1	
Systematic name	Glutathione:dehydroascorbate oxidoreductase	
Recommended name	Glutathione dehydrogenase (ascorbate)	
Other names	Dehydrogenase, glutathione (ascorbate); Dehydroascorbic reductase; Dehydroascorbic acid reductase; Glutathione dehydroascorbate reductase; DHA reductase; Dehydroascorbate reductase; GDOR; Glutathione:dehydroascorbic acid oxidoreductase	
CAS registration no.	9026-38-4	
Reaction catalysed	2 Glutathione + dehydroascorbate = oxidized glutathione + ascorbate (ordered or random mechanism involving the formation of a ternary complex , zero-ordered kinetics only observed for hydrogen acceptor, not for glutathione)	
Reaction type	Redox reaction	
Substrate spectrum	**1.**	Dehydroascorbic acid + glutathione (specific for glutathione as hydrogen donor, specific for dehydroascorbate , L-threo-dehydroascorbate: best substrate , D-threo-dehydroascorbate: 20% of the activity with L-threo-dehydroascorbate, activity with both erythro-dehydroascorbates lies between the threo-stereoisomers
	2.	More (L-cysteinyl-L-glycine not active as hydrogen donor, NADH, NADPH and cysteine cannot substitute for glutathione, cysteine cannot replace glutathione)
Products	**1.**	L-Ascorbate + oxidized glutathione
	2.	?
Natural substrates	Dehydroascorbic acid + glutathione (vitamin C-conserving mechanism, regeneration of ascorbate from monodehydroascorbate and dehydroascorbate produced by ascorbate peroxidase, EC 1.11.1.11, for scavenging hydrogen peroxide)	
Turnover number		
Specific activity	0.264; More (assay method , spectrometric assay)	
Km value	1.3 (dehydroascorbate); 3.8 (glutathione); 0.85 (glutathione); 0.26 (dehydroascorbate) ; 0.34 (dehydroascorbate); 4.45 (glutathione) ; 0.39 (dehydroascorbate); 4.35 (glutathione);	
pH optimum	7.0 (maximal activity above); 7.5 ; 8 ; More (nonenzymatic reduction occurs rapidly at pH 7.5-8.0, therefore the assay should be performed between pH 6.3 and 6.8)	
pH range		
Temperature optimum	38	
Temperature range		
Cofactors/prosthetic groups	2-Mercaptoethanol (1 mM, stimulation); Dithiothreitol (1 mM, stimulation)	
Metal ions/salts		

Inhibitors	Zn2+ ; Fe3+ ; Cu2+ ; Co2+ ; N-Ethylmaleimide ; p-Chloromercuribenzoate; Dehydroascorbate (high concentration); p-Hydroxymercuribenzoate ; Iodoacetic acid ; HgCl2; Mersalyl
Organism (original)	Euglena gracilis Z ; Wheat; Guinea pig ; Spinach ; Human ; Rat; Potato
Source	Blood neutrophile; Lymphocytes; Seeds (germinating); Brain; Adrenal ; Stomach; Liver ; Leaves; Wheat flour; Tubers ; Erythrocytes
Localization	Cytoplasm
Purification	Euglena gracilis Z (partial); Spinach (partial); Wheat ; Potato
Crystallization	-
Molecular Weight	23000 (potato, gel filtration); 24000 (wheat); 25000 (spinach, gel filtration); 28000 (Euglena gracilis Z, gel filtration)
Subunits	
Carbohydrate/Lipid	-
Cross-refs to NBRF/PIR	
Cloned	-
Cross-refs to Brookhaven	
pH stability	7.0-8.0 (stable up to 42°C)
Temperature stability	42 (stable up to, pH 7.0-8.0); 50 (10 min, 35% loss of activity, 7 min, stable up to); 60 (10 min, complete inactivation)
Organic solvent stability	
Oxidation stability	
General stability	Purified dehydroascorbate reductase is extremely unstable
Storage	4°C, in presence of 2-mercaptoethanol stable for several days

stability		
Renaturated	-	
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