Kits for Oxidative Stress & Aging Research

Vitamin C Assay Kit

Useful for the measurement of total vitamin C (AsA + DHAsA)

Background and Features

Vitamin C (L-Ascorbic acid) is a water-soluble vitamin with strong reducing action and is an important coenzyme for internal hydroxylation reactions (e.g. collagen). Vitamin C is present in both a reduced form (ascorbic acid (AsA)) and an oxidized form (dehydroascorbic acid (DHAsA)). This Vitamin C Assay Kit measures total vitamin C (i.e., AsA + DHAsA).

Daniel W.B., Gladys E, James E.M. : *Clinica Chimica Acta*, **44**, 47-52 (1973)

Application

Vitamin C (L-ascorbic acid) in internal organ, tissue, plasma, fruit and Vegetables (Tomato, Lettuce) can be measured.

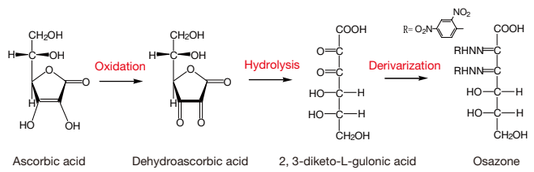
Kit Components

[Sufficient for 100 assay points]
*This kit is sufficient for 100 assay points (50 samples, duplicate assay points).

- Reagent①
  Oxidizing agent — 1 vial (2 ml)
- Reagent②
  5% Metaphosphoric acid/2% $SnCl_2$ — 1 vial (10 ml)
- Reagent③
  2,4-Dinitrophenylhydrazine (DNPH) — 1 vial (Dissolve in 3 ml 44% sulfuric acid)
- Reagent④
  5% Metaphosphoric acid — 1 vial (10 ml)
- Vitamin C Standard Stock Solution — 1 vial (1 ml)

Principle of measurement

For each sample, reduced vitamin C (AsA) is converted to DHAsA by Reagent 1, an oxidizing agent. Vitamin C is then derivitized with DNPH. Total vitamin C concentration (AsA + DHAsA) is then determined by measuring UV absorbance of the DNPH derivative.



Ascorbic acid → Dehydroascorbic acid → 2, 3-diketo-L-gulonic acid → Osazone

Example Experiment

Vitamin C Assay Report

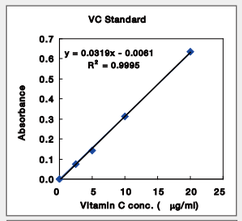
Sample A : Glucose solution (Glucose conc. 0.1～100 mg/ml)
Sample B : Vitamin C + Glucose solution (Vitamin C conc. 10 mg/ml + Glucose conc. 0～100 mg/ml)
Used Kit : Vitamin C Assay Kit (SHIMA LABORATORIES)

Methods

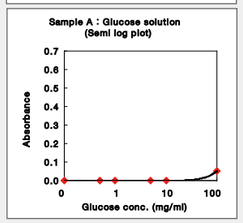
1 Preparation of Glucose solution (Glucose conc. 0.1～100 mg/ml)
1-1. 1 g Glucose was dissolved in 10 ml of 5% Metaphosphoric acid solution (MPA). (Glucose conc. 100 mg/ml)
1-2. 100 mg/ml Glucose solution was diluted in 5% MPA to 0.1～10 mg/ml Glucose solution. (See table 1.1.2)

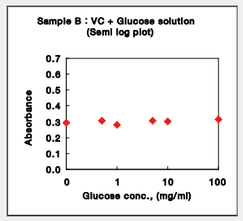
2 Preparation of Vitamin C + Glucose solution (Vitamin C conc. 10 mg/ml + Glucose conc. 0～100 mg/ml)
2-1. 1 g Vitamin C was dissolved in 10 ml of 5% MPA. (Vitamin C conc. 100 mg/ml)
2-2. Furthermore, 100 mg/ml Vitamin C solution was diluted in 5% MPA to 10 mg/ml Vitamin C solution.
2-3. 1 g Glucose was dissolved in 10 ml of 10 mg/ml Vitamin C solution. (Vitamin C conc. 10 mg/ml, Glucose conc. 100 mg/ml)
2-4. 10 mg/ml Vitamin C + 100 mg/ml Glucose solution was diluted in 10 mg/ml Vitamin C solution. (See table 2.2.4)

3 After preparation of samples, the measurement of Vitamin C was performed using Vitamin C Assay Kit according to the manufacture's instruction.



VC 10 mg/ml
Absorbance 0.31





In this report, detection of glucose and vitamin C by the Vitamin C assay kit was evaluated. Glucose solution (Sample A) and vitamin C + glucose solution (Sample B) were used for the samples. The sample of glucose concentration was 10 times higher than Vitamin C concentration.

Results:
The Vitamin C Assay kit did not detect samples containing up to 10 mg/ml glucose solution and reacted only slightly with 100 mg/ml glucose (sample A). Vitamin C was detected at 10 mg/ml in the presence of up to 100 mg/ml glucose (sample B) with no increase in signal at the highest glucose concentration. Thus, The Vitamin C Assay kit specifically detects vitamin C with little or no cross reaction with glucose.

Product Name	Cat#	Quantity
Vitamin C, Assay kit	SML-ROIK02-EX	1 kit

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COSMO BIO CO., LTD.

Oxidative Stress & Aging Research Kits

- SMP-30/Gluconolactonase (GNL) Western Blot Kit
- Protein Carbonyls Western Blot Detection Kit
- Protein Carbonyls Immunohistochemical Staining Kit
- Vitamin C Assay Kit

SMP-30/Gluconolactonase (GNL) Western Blot Kit

Aging, Vitamin C and Lung Disease Research Kit

Background

SMP30 (Senescence Marker Protien-30) is a 34 kDa protein found in kidney and lung at levels that diminish with age[1,2]. It is also expressed in brain, adrenals, stomach, ovary, uterus, testis and epidermis. SMP-30 functions to catalyze lactone hydrolysis and is a key enzyme in mammalian vitamin C (L-ascorbic acid) biosynthesis[3]. In fact, SMP-30 knockout mice displayed symptoms of scurvy when fed a vitamin C diet. Moreover, SMP-30 protects mouse lung from oxidative stress associated with aging and smoking[4].

*SMP30, Gluconolactonase, and Regucalcin refer to the same protein.

Kit Components

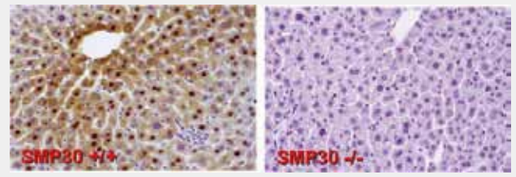
- Antibody : Rabbit anti SMP30/GNL antibody (0.1 mL)
  *This kit does not contain NaN_3
- Specimen : SMP30/GNL Knockout Mouse Liver (2 slides)
  Wild type Mouse Liver (2 slides)
- Tissue extract : SMP30/GNL Knockout Mouse Liver
  (30 μL, Protein concentration 0.4 mg/mL)
  Wild type Mouse Liver
  (30 μL, Protein concentration 0.4 mg/mL)

References

1. Ishigami, A. et al., Senescence marker protein-30 knockout mouse liver are highly susceptible to TNF-alpha- and Fas-mediated apoptosis. *Am. J. Pathol.* **161** 1273-1281 (2002)
2. Ishigami, A. et al., Nuclear localization of senescence marker protein-30 (SMP30) in cultured mouse hepatocytes and its homology to RNA polymerase. *Biosci. Biotechnol. Biochem.* **67** 158-160 (2003)
3. Kondo, Y. et al., Senescence Marker Protein 30 Functions as Gluconolactonase in L-Ascorbic Acid Biosynthesis and Its Knockout Mice Are Prone to Scurvy. *Proc. Nat. Acad. Sci.* USA **103** 5723-5728 (2006)
4. Sato, T. et al., Senescence Marker Protein-30 Protects Mice Lungs from Oxidative Stress, Aging and Smoking. *Am. J. Respir. Crit. Care Med.* **174** 530-537 (2006)

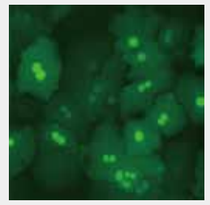
Example Experiment

Immunohistochemical staining



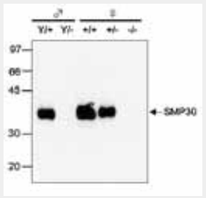
Mouse liver stained with SMP30/GNL antibody at 1:300 dilution and developed by 3,3'-diaminobenzidine. Nucleus and cytoplasm of wild type (SMP30+/+) mice stained, but not stained in liver from SMP30 knockout (SMP30-/-) mice.

Immunofluorescence staining



Primary cultured mouse hepatocytes stained with SMP30/GNL antibody at 1:200 dilution. Nucleus and cytoplasm stained in green.

Western Blot Analysis



Male SMP-30 Wild Type Mouse: Y/+
Male SMP-30 Knockout Mouse: Y/-
Female SMP-30 Wild Type Mouse: +/+
Female SMP-30 Heterozygous Mouse: +/-
Female SMP-30 Knockout Mouse: -/-

Liver protein extracts from male and female mice were separated by gel electrophoresis and western blotting was performed using SMP-30/GNL antibody at 1:1000 dilution.

Product Name	Cat#	Quantity
SMP30 [Gluconolactonase, GNL] Western Blot & Immunostain Kit	SML-ROIK01-EX	1 kit
Anti rat SMP30 [Regucalcin, Gluconolactonase (GNL)] polyclonal antibody	SML-ROI001-EX	0.1 ml
Anti human PAD2 polyclonal antibody	SML-ROI002-EX	0.1 ml
Anti human GFAP polyclonal antibody	SML-ROI003-EX	0.2 ml

Protein Carbonyls Western Blot Detection Kit

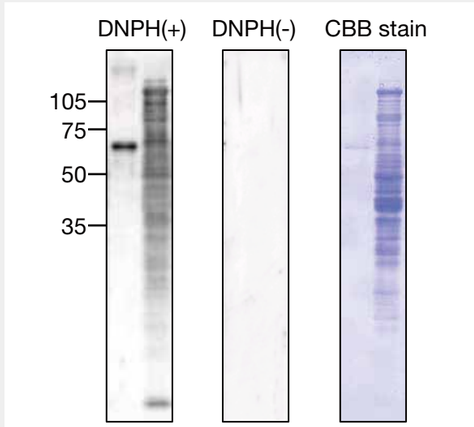
Improved immunodetection kit for studying the mechanisms and role of oxidative stress in health and disease

Background and Features

Reactive oxygen species (ROS) are produced as a result of normal cell metabolism or by exposure to ionizing radiation, chemicals, or other environmental stress. ROS are well known to promote non-specific protein oxidation with subsequent negative effects on protein structure and function. Typical ROS-induced protein modifications include the transformation of lysine, arginine, proline, and threonine side chain amines into carbonyls. The chemical stability of these carbonyl derivatives allows their detection and quantification, providing a sensitive and reliable marker of ROS-mediated protein oxidation.

With the Protein Carbonyls Western Blot Detection Kit, carbonyl groups are specifically detected by Western blotting using an anti-dinitrophenyl (DNP) antibody following modification of protein carbonyls with 2,4-dinitrophenylhydrazine (DNPH). In contrast to older methods where DNP modification is performed before protein electrophoresis, an advantageous feature of this improved kit is that DNP modification is performed after protein transfer to the blotting membrane. As DNP derivatization alters electrophoretic properties of proteins, derivatization after membrane transfer facilitates and simplifies the identification of individual carbonyl-modified proteins. Further, protocol times using this improved technique are significantly shortened.

Example Experiment



<Western Blot Analysis>
★DNP antibody at 1:2,000 dilution used
DNPH(+) : DNPH in 2 N HCl
Left lane:oxidized BSA 0.1 g
Right lane:mouse liver extract 5 g
DNPH(-) : 2 N HCl
Left lane:oxidized BSA 0.1 g
Right lane:mouse liver extract 5 g
CBB stain : proteins stained by Coomassie brilliant blue

*The membrane was incubated with (DNPH(+)) or without (DNPH(-)) DNPH solution. Protein carbonyls were detected only DNPH-treated membrane but not detected at all untreated membrane.
[Data from Dr. Akihito Ishigami, Tokyo Metropolitan Institute of Gerontology]

Kit Components

- Antibody :
  Rabbit anti-DNP antibody (0.075 mL)
  *This kit does not contain NaN_3
- DNPH solution(shade the light):
  10X 2,4-Dinitrophenylhydrazine (DNPH) solution (15 mL)
- Oxidized protein :
  oxidized BSA , soluble in SDS-PAGE sample buffer[2] (0.15 mL)

References

1. Nakamura A. et al., Analysis of protein carbonyls with 2,4-dinitrophenyl hydrazine and its antibodies by immunoblot in two-dimensional gel electrophoresis. *J Biochem* (Tokyo). **119** 768-774 (1996)
2. Goto S. et al., Age-associated, oxidatively modified proteins: *A critical evaluation. Age* **20** 81-89 (1997)
3. Goto S. et al., Carbonylated Proteins in Aging and Exercise: Immunoblot Approaches. *Mech Ageing Dev* **107** 245-253 (1999)
4. Nakamura A. et al., Vitellogenin-6 is a major carbonylated protein in aged nematode, Caenorhabditis elegans. *Biochem Biophys Res Commun.* **264** 580-583 (1999)
5. Robinson CE. et al., Determination of protein carbonyl groups by immunoblotting. *Anal Biochem.* **266** 48-57 (1999)
6. Sato T. et al., Senescence marker protein-30 protects mice lungs from oxidative stress, aging, and smoking. *Am J Respir Crit Care Med.* **174** 530-537 (2006)

Product Name	Cat#	Quantity
Protein carbonyls western blot detection kit (15 Blots)	SML-ROIK03-EX	1 kit

Protein Carbonyls Immunohistochemical Staining Kit

for the specific detection of protein oxidation by ROS.

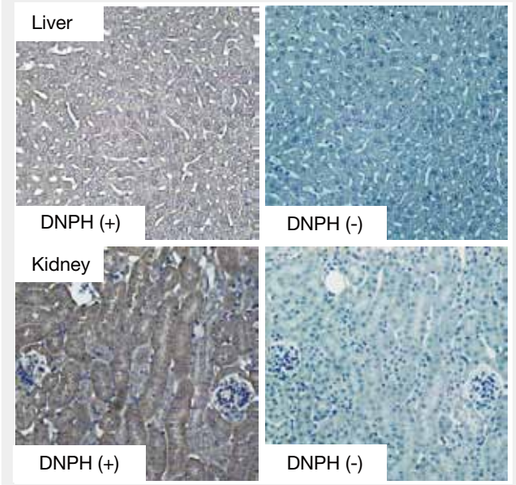
Novel kit research in aging, oxidative stress and oxidative stress related disease

Background and Features

Reactive oxygen species (ROS) are produced as a result of normal cell metabolism or by exposure to ionizing radiation, chemicals, or other environmental stress. ROS are well known to promote non-specific protein oxidation, with negative effects protein structure and function. Typical ROS-induced protein modifications include the transformation of lysine, arginine, proline, and threonine side chain amines into aminoacyl carbonyls. The chemical stability of these carbonyl derivatives allows their detection and quantification, providing a sensitive and reliable marker of ROS-mediated protein oxidation.

With the Protein Carbonyls Immunohistochemical Staining Kit, carbonyl groups in tissue are first derivitized by reaction with 2,4-Dinitrophenylhydrazine (DNPH). DNPH-derivitized carbonyls are then detected using an anti-DNP specific antibody suitable for immunohistochemical procedures. This novel kit is the first to enable detection of protein carbonyls by immunohistochemical staining. A detection kit for protein carbonyls in cellular lysates by Western blotting with anti-DNP antibody is also available (Cat No.SML-ROIK03-EX).

Example Experiment



Mouse liver and kidney sections stained with rabbit anti-DNP antibody at 1:100 dilution and developed by 3,3'-diaminobenzidine (DAB). Specimens were incubated with (DNPH(+)) or without (DNPH(-)) DNPH solution. Protein carbonyls are detected only in DNPH-treated specimen and not detected at all in untreated specimen.
Courtesy of Dr. Akihito Ishigami, Tokyo Metropolitan Institute of Gerontology

Kit Components

- Antibody : Rabbit anti-DNP for immunohistochemistry (0.06 mL)
  *This kit does not contain NaN_3
- Control Specimen : Mouse Kidney
  (Methacarn fixed paraffin-embedded sections) (2 slides)
- DNPH solution : 2,4-Dinitrophenylhydrazine (DNPH) solution (6 mL)

References

1. Nakamura A. et al., Analysis of protein carbonyls with 2,4-dinitrophenyl hydrazine and its antibodies by immunoblot in two-dimensional gel electrophoresis. *J Biochem* (Tokyo). **119** 768-774 (1996)
2. Goto S. et al., Age-associated, oxidatively modified proteins: *A critical evaluation. Age* **20** 81-89 (1997)
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6. Sato T. et al., Senescence marker protein-30 protects mice lungs from oxidative stress, aging, and smoking. *Am J Respir Crit Care Med.* **174** 530-537 (2006)

Product Name	Cat#	Quantity
Protein Carbonyls Immunohistochemical Staining Kit (50 slides)	SML- ROIK04-EX	1 kit