

Oxidative Stress Markers

Measurement Kit / Antibody / Anti Oxidant Assay Kit

Protein Oxidation Markers

▶ Dityrosine (DT) ELISA Kit & antibody



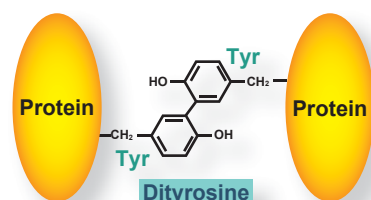
A stable marker for protein oxidation

Protein tyrosine residues modified by free radicals or oxidative stress represent a major form of protein oxidation. Oxidized tyrosine derivatives found in proteins include nitrotyrosine, halogenated tyrosine, and dityrosine. Dityrosine (DT) is a tyrosine dimer that results from tyrosyl radicals formed by reactive oxygen species (ROS), metal-catalyzed oxidation, ultraviolet irradiation, and peroxidases. It is a specific marker of protein oxidation. DT residues have been found in atherosclerotic lesions and lipofuscin of pyramidal neurons from aged human brains.

Dityrosine ELISA – suitable for urine samples

Monitoring dityrosine levels in urine samples is a non-invasive route to assess oxidative stress *in vivo*. This convenient ELISA Kit has been developed to give accurate results with urine samples.

Description	Cat. No.	Size	Measuring Range	Sample
Dityrosine (DT) ELISA Kit	NNS-KDT-010E-EX	96 well	0.3 - 10 µmol/L	urine



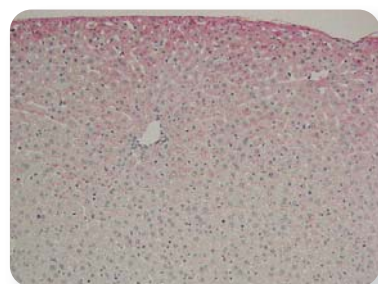
Dityrosine monoclonal antibody for immunohistochemistry and western blotting

This antibody is specific for free dityrosine, 3-(p-hydroxyphenyl) propionic acid dimer, dityrosine-BSA conjugate, and dityrosine in other proteins or peptides. Cross reactivity has been tested against the following analogues: Nitrotyrosine, chlorotyrosine, aminotyrosine, phosphotyrosine, tyrosine, 3-(p-hydroxyphenyl) propionic acid, phenylalanine, 3,4-dihydroxyphenylacetic acid (DOPA).

Reference: Y Kato, *et al.*, *Biochem Biophys Res Commun.* **275** (1), p11-15 (2000)

Description	Cat. No.	Size	Clone	Host	Application
Anti Dityrosine (DT) monoclonal antibody	NNS-MDT-020P-EX	20 µg	1C3	Mouse	IHC / WB

▶ Anti Dibromo Tyrosine monoclonal antibody



A new biomarker of protein oxidation due to granulocyte activity

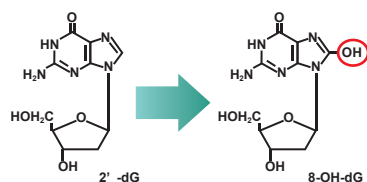
Neutrophils and eosinophils play important roles in the defense against microbial infection. Myeloperoxidase and eosinophil peroxidase present in these cell types are known to catalyze the formation of hypochlorous acid (HOCl) and hypobromous acid (HOBr). These reactive intermediates react with proteins and are known to form tyrosine halogenations including dibromotyrosine (DiBrY). Anti-DiBrY mouse mab 3A5 is specific for 3,5-DiBrY, and is suitable for immunohistochemical analysis of oxidative stress.

Reference: Y Kato, *et al.*, *Free Rad Biol Med*, **38**, p24-31 (2005)
Wu W, Chen Y, *et al.*, *Biochemistry*. **38** (12): 3538-48 (1999)

Description	Cat. No.	Size	Clone	Host	Application
Anti DiBrY monoclonal antibody	NNS-MBY-020P-EX	20 µg	3A5	Mouse	IHC

DNA Oxidation Markers

▶ 8-OHdG (8-hydroxy-2'-deoxyguanosine) ELISA Kit



Formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) by oxygen radical.
H Kasai: *Environmental Mutagen Research*. Vol. 10. p73-78 (1988)



Oxidative stress is known to play an important role in the development of various diseases and aging processes.

8-OHdG is formed when DNA is oxidized by reactive oxygen species (ROS). 8-OHdG is one of the most sensitive biomarkers for oxidative stress and can be detected in urine, serum, and tissue DNA from humans and animals.

High specificity and high sensitivity

The cross-reactivity of this assay kit has been characterized against 19 8-OHdG analogs.

Easy, fast operation

Assays are completed in 3.5 hours (NNS-KOG-200SE-EX). Requires no sample pretreatment or highly specialized instrumentation. Read on standard microplate reader at 450 nm.

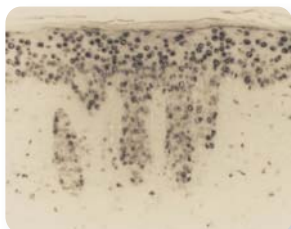
Reference: S Saito, *et al.*, *Res. Commun. Mol. Pathol. Pharmacol.* **107** (1&2), p39-44 (2000)
[Development and assessment of 8-OHdG Check ELISA.]

Nagayoshi Y, *et al.*, *Am J Cardiol.* **95** (4), p514-517 (2005)
[Measurement of 8-OHdG in human urine.]

Morillas-Ruiz J, *et al.*, *Eur J Appl Physiol* **95** (5-6), p543-549 (2005)
[Study about exercise, antioxidant supplements and urinary 8-OHdG.]

Description	Cat. No.	Size	Measuring Range	Sample
8-OHdG Check ELISA Kit				
	NNS-KOG-200SE-EX	96 well	0.5 - 200 ng/mL	urine
Highly Sensitive 8-OHdG Check ELISA Kit				
	NNS-KOG-HS10E-EX	96 well	0.125 - 10 ng/mL	serum and tissue DNA

▶ Anti 8-OHdG monoclonal antibody



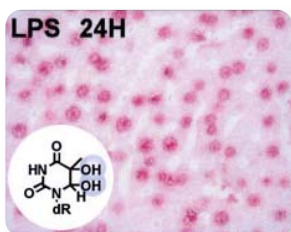
Highly specific for 8-OHdG

The cross-reactivity of anti 8-OHdG mouse mab N45.1 (IgG1,κ), has been characterized against 19 8-OHdG analogues including dA, dC, dT, 2'-dG, 8-OHdA, 2'-dI, 2'-dU, O6-methyl-dG, G, 7-methyl-G, 8-Br-G, 8-sulfhydryl-G, 6-SH-G, 8-OH-G, Gua, O6-methyl-Gua, 8-OH-Gua, uric acid, urea, creatinine, creatine (G: Guanosine, Gua: Guanine).

Reference: S Toyokuni, *et al.*, *Lab. Invest.* **76** (3), p365-374 (1997)

Description	Cat. No.	Size	Clone	Host	Application
Anti 8-OHdG monoclonal antibody					
	NNS-MOG-020P-EX	20 µg	N45.1	Mouse	IHC
	NNS-MOG-100P-EX	100 µg	N45.1	Mouse	IHC

▶ Anti Thymidine Glycol (TG) monoclonal antibody



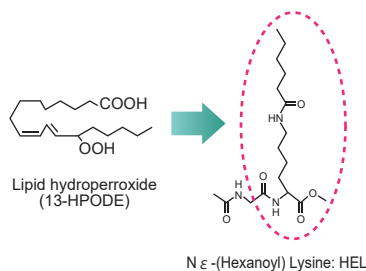
Biomarker for oxidative DNA damage

Thymidine glycol (TG) is formed by hydroxyl radical oxidation of thymidine and is another biomarker of oxidative stress. Anti-TG mouse mab 2E8 is specific for TG in DNA polymers and suitable for sensitive detection of TG by immunohistochemistry.

Description	Cat. No.	Size	Clone	Host	Application
Anti TG monoclonal antibody					
	NNS-MTG-100P-EX	100 µg	2E8	Mouse	IHC

Lipid oxidation Markers

▶ Hexanoyl-Lysine adduct (HEL) ELISA Kit



A new biomarker of lipid peroxidation

Hexanoyl-Lysine adduct (HEL) is a novel lipid hydroperoxide-modified lysine residue. HEL is formed by lipid peroxidation of C6 fatty acids such as linoleic acid or arachidonic acid. HEL may be a useful biomarker for early stage lipid peroxidation and is detected in oxidatively modified LDL, in human atherosclerotic lesions and in human urine.

HEL ELISA Kit – suitable for urine and serum samples

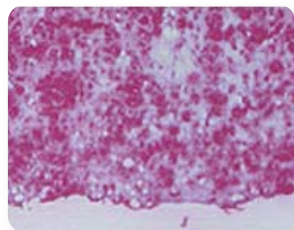
A convenient, easy to use kit with photometric readout (450 nm), suitable for urine and serum from humans and other animals. Assay Range: 2-700 nmol/L.

Reference: Rummenie VT, *et al.*, *Cytokine* **43** (2), p200-208 (2008).
Geyama Y, *et al.*, *Rheumatol Int* **28** (3), p245-251 (2008).
Ryo K, *et al.*, *Pathobiology* **73** (5), p252-260 (2006).



Description	Cat. No.	Size	Measuring Range	Sample
Hexanoyl-Lys adduct (HEL) ELISA Kit	NNS-KHL-700E-EX	96 well	2 - 700 nmol/L	urine, serum, cultured cells and other biological materials

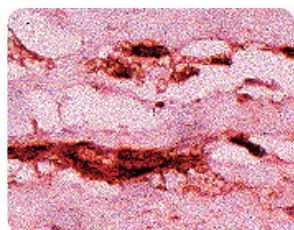
▶ Anti Hexanoyl-Lysine (HEL) monoclonal antibody



The cross reactivity of Anti HEL mouse mab 5F12 (IgG, κ) has been characterized against 8 HEL analogues including MDA, glyoxal, methyl glyoxal, 1-hexanal, 2-hexenal, 1-nonanal, 2-nonenal and 4-hydroxy-2-nonenal. Presented as lyophilized powder.

Description	Cat. No.	Size	Clone	Sample
Anti HEL monoclonal antibody	NNS-MHL-021P-EX	20 μ g	5F12	Mouse

▶ Anti Methylglyoxal (MG) monoclonal antibody

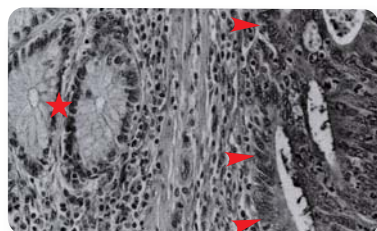


Methylglyoxal (MG) is an endogenous intermediate that increases in diabetic patients. MG is a common Maillard reaction intermediate (glycation) that reacts with proteins to form advanced glycation end products (AGE). MG reacts with arginine residues in protein to form numerous adducts including argpyrimidine. Anti-MG mouse mab clone 3C is specific for argpyrimidine.

Reference: Uchida K, *et al.*, *Invest Ophthalmol Vis Sci.* **42** (6), p1299-1304. (2001)

Description	Cat. No.	Size	Clone	Sample
Anti MG monoclonal antibody	NOF-N213430-EX	30 μ g	3C	Mouse

▶ Anti 4-HNE monoclonal antibody

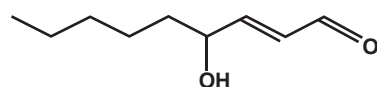


Immunohistochemistry of colorectal carcinoma cells. Adenocarcinoma (arrows) and nontumorous epithelial cells (star; \times 240). (S. Kondo *et al.* *Free Radical Biology & Medicine* **27** pp401-410, 1999)

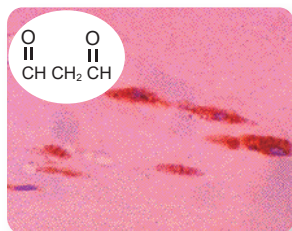
Highly specific for 4-HNE

Membrane lipids are included in the targets of reactive oxygen species (ROS). 4-hydroxy-2-nonenal (4-HNE) is a major product of membrane lipid peroxidation. Mouse mab anti 4-HNE (clone HNE-J2, IgG1, κ) is highly specific for 4-HNE-His/Lys/Cys adducts. Presented as a lyophilized powder.

Description	Cat. No.	Size	Clone	Host	Application
Anti 4-HNE monoclonal antibody	NNS-MHN-020P-EX	20 μ g	HNE-J2	Mouse	IHC / WB
	NNS-MHN-100P-EX	100 μ g	HNE-J2	Mouse	IHC / WB



▶ Anti Malondialdehyde (MDA) monoclonal antibody

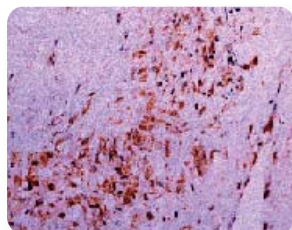


A new biomarker of lipid peroxidation

Malondialdehyde (MDA) is one of the major aldehydes derived from lipid peroxidation. Anti-MDA mab 1F83 is specific for MDA modified proteins and is suitable for immunohistochemistry and western blotting.

Description	Cat. No.	Size	Clone	Host	Application
Anti MDA monoclonal antibody	NOF-N213530-EX	30 µg	1F83	Mouse	IHC / WB

▶ Anti Acrolein (ACR) monoclonal antibody

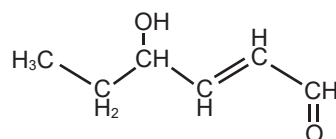


Acrolein (ACR) is a representative carcinogenic aldehyde found ubiquitously in the environment. ACR also forms in cells through oxidation reactions including lipid peroxidation and myeloperoxidase-catalyzed amino acid oxidation. ACR is highly reactive with lysine residues in protein. The cross reactivity of anti-ACR mouse mab 5H4 has been characterized against 14 analogues including crotonal, 2-hexenal, 2-octanal, 2-nonenal, 4-decanal, 2,4-decadienal, MDA, 4-HHE, 4-HPE, 4-HON, 4-HNE, n-propanal, n-pentanal and n-hexenal.

Reference: K.Satoh, *et al.*, *Analytical Biochemistry*, **270**, p323-328 (1999)

Description	Cat. No.	Size	Clone	Host	Application
Anti ACR monoclonal antibody	NOF-N213320-EX	20 µg	5H4	Mouse	IHC / WB
	NOF-N213310-EX	100 µg	5H4	Mouse	IHC / WB

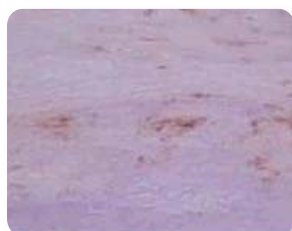
▶ Anti 4-HHE monoclonal antibody



The aldehyde 4-hydroxy-2-alkenal, a major product of lipid peroxidation, is formed by peroxidation of n-3 fatty acids such as docosahexaenoic acid. HHE exhibits several biological effects including high toxicity. HHE aldehyde is highly reactive with protein histidine residues, forming Michael-addition type adducts. Anti 4-HHE mouse mab HHE53 (IgG1,κ) is specific for HHE-histidine Michael adduct (HHE-His), and enables detection of HHE-His in tissue samples.

Description	Cat. No.	Size	Clone	Host	Application
Anti 4-HHE monoclonal antibody	NOF-N213730-EX	30 µg	HHE53	Mouse	IHC

▶ Anti 7-KC monoclonal antibody

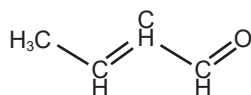


Cholesterol oxidation products, especially 7-Ketocholesterol (7KC), have been the focus of much attention due to their presence in human atherosclerotic plaques. Further, cholesterol oxidation products display a wide range of atherogenic properties *in vitro* and to some extent, *in vivo*. Anti-7-KC mouse mab 35A is specific for 7KC and can be used to detect 7KC in tissue samples. Frozen tissue samples are recommended for immunohistochemistry with this antibody.

* Immunohistochemistry ; It is recommended that the antibody be tried at 5-10 µg/mL on frozen tissue. Not applicable to paraffin embedded tissues.

Description	Cat. No.	Size	Clone	Host	Application
Anti 7-KC monoclonal antibody	NOF-N213820-EX	20 µg	35A	Mouse	IHC
	NOF-N213810-EX	100 µg	35A	Mouse	IHC

▶ Anti CRA monoclonal antibody



Crotonaldehyde (CRA) is a representative carcinogenic aldehyde formed in cells through lipid peroxidation. CRA is highly reactive with protein lysine residues leading to the formation of numerous adducts. Anti CRA mouse mab 82D3 reacts specifically with proteins modified by CRA.

Reference: K Ichihashi, *et al.*, *J Biol Chem* **276** (26), p23903-23913 (2001)

Description	Cat. No.	Size	Clone	Host	Application
Anti CRA monoclonal antibody	NOF-N213630-EX	30 µg	82D3	Mouse	IHC

Oxidative Stress Markers

Selection Guide for Oxidative Stress Markers

Type	Description	Spec	Application			Remark
			Urine	Serum	Tissue	
DNA oxidation	8-OHdG Check ELISA Kit #NNS-KOG-200SE-EX, 96 wells	Measuring range: 0.5 - 200 ng/mL	OK	OK (animals)	-	Suitable for urine samples. Assay time: approx 3.5 hours.
	Highly Sensitive 8-OHdG Check ELISA Kit #NNS-KOG-HS10E-EX, 96 wells	Measuring range: 0.125 - 10 ng/mL	OK	OK (human)	OK	Suitable for human serum, tissue and cultured cells. Assay time: overnight and 2 hours.
	Anti 8-OHdG monoclonal antibody (N45.1) #NNS-MOG-020P-EX / #NNS-MOG-100P-EX	20 µg / 100 µg of IgG	-	-	OK	Suitable for immunohistochemistry and ELISA.
	Anti Thymidine Glycol (TG) monoclonal antibody #NNS-MTG-100P-EX	100 µg of IgG	-	-	OK	Suitable for immunohistochemistry.
Lipid oxidation	Hexanoyl-Lysine (HEL) ELISA Kit #NNS-KHL-700E-EX, 96 wells	Measuring range: 2 - 700 nmol/L	OK	OK	OK	New biomarker for early stage of lipid oxidation. Suitable for urine, serum cultured cells and other biological materials.
	Anti Hexanoyl-Lysine (HEL) monoclonal antibody #NNS-MHL-021P-EX	20 µg of IgG	-	-	OK	Suitable for immunohistochemistry, western blotting and ELISA.
	Anti 4-HNE monoclonal antibody (HNEJ2) #NNS-MHN-020P-EX, #NNS-MHN-100P-EX	20 µg / 100 µg of IgG	-	-	OK	Suitable for immunohistochemistry and western blotting.
	Anti Acrolein (ACR) monoclonal antibody (5F6) #NOF-N213320-EX, #NOF-N213310-EX	20 µg / 100 µg of IgG	-	-	OK	Suitable for immunohistochemistry, western blotting and ELISA.
	Anti MDA monoclonal antibody (1F83) #NOF-N213530-EX	30 µg of IgG	-	-	OK	Suitable for immunohistochemistry and western blotting.
	Anti 4-hydroxy-2-hexenal (4-HHE) monoclonal antibody #NOF-N213730-EX	30 µg of IgG	-	-	OK	Suitable for immunohistochemistry.
	Anti Crotonaldehyde (CRA) monoclonal antibody #NOF-N213630-EX	30 µg of IgG	-	-	OK	Suitable for immunohistochemistry.
	Anti Methylglyoxal (MG) monoclonal antibody #NOF-N213430-EX	30 µg of IgG	-	-	OK	Suitable for immunohistochemistry.
	Anti 7-ketocholesterol (7-KC) monoclonal antibody #NOF-N213820-EX, #NOF-N213810-EX	20 µg / 100 µg of IgG	-	-	OK	Suitable for immunohistochemistry.
Protein oxidation	Dityrosine (DT) ELISA Kit #NNS-KDT-010E-EX, 96 wells	Measuring range 0.3 - 10 µmol/L	-	-	OK	Suitable for urine samples.
	Anti Dityrosine (DT) monoclonal antibody #NNS-MDT-020P-EX	20 µg of IgG	-	-	OK	Suitable for immunohistochemistry and western blotting.
	Anti Dibromo-Tyrosine (DiBrY) monoclonal antibody #NNS-MBY-020P-EX	20 µg of IgG	-	-	OK	Suitable for immunohistochemistry and western blotting.
Anti oxidant assay	Test Kit for Potential Antioxidant(PAO) Kit #NNS-KPA-050E-EX, 96 wells	Measuring range: 21.9 - 4378 µmol/L	-	OK	beverage	Suitable for serum, foods, beverage and antioxidants. Can detect both hydrophobic and hydrophilic antioxidants.

Anti Oxidant Assay Kit

▶ Test Kit for Total Potential Antioxidant (PAO)



Significance of antioxidants

For accurate assessment of oxidative stress, it may be essential to consider not only the measurement of ROS and oxidative damage, but also antioxidant activity. Recently, ROS-scavaging antioxidants in foods have attracted a great deal of attention. The PAO Assay Kit offers a fast (5 min), easy and convenient method to measure the antioxidant capacity in samples, utilizing the reduction of cupric ion ($\text{Cu}^{++} \rightarrow \text{Cu}^+$).

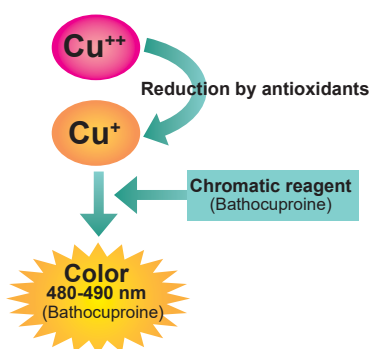
Application to foods, beverages and serum

PAO can detect not only hydrophilic antioxidants such as Vitamin C, glutathione, but also can detect hydrophobic antioxidants such as Vitamin E. Applicable for assessment of total antioxidants of serum, foods and beverage samples.

Useful for foods, beverages and serum

The PAO Assay Kit can detect both hydrophilic antioxidants such as Vitamin C and glutathione as well as hydrophobic antioxidants such as Vitamin E. Applicable for assessment of total antioxidant in food and beverage samples, and human and animal sera. The assay readout is photometric (492 nm), and requires no highly specialized equipment.

Reference: C. VASSALLE, *et al.*, *Journal of Internal Medicine*. **256**, p308–315 (2004)



Samples	Sample pretreatment		Sample required	Assay example	
	Pre-dilution	Assay dilution			
Human serum or heparinized plasma	-	Mix with 39 volumes of sample diluent	10 μL	Mean 1069 \pm 145 $\mu\text{mol/L}$	
	Mix with 3 volumes of distilled water				
Human urine	Mix with 3 volumes of distilled water		2 μL	5508 $\mu\text{mol/L}$	
Foods	Red wine		Mix with 3 volumes of distilled water	2 μL	45479 $\mu\text{mol/L}$
	Green tea		Mix with 7 volumes of distilled water	2 μL	8728 to 46687 $\mu\text{mol/L}$
	Black tea	Mix with 7 volumes of distilled water	2 μL		
	Coffee	Mix with 27 volumes of distilled water	1 μL		

Description	Cat. No.	Size	Measuring Range
Potential Anti Oxidant (PAO) Kit	NNS-KPA-050E-EX	96 well	21.9 - 4378 $\mu\text{mol/L}$ (antioxidant power)

▶ Related Products

Description	Cat. No.	Size
Vitamin C (L-ascorbic acid) measurement in tissue, plasma, fruit and vegetable. Vitamin C Assay Kit	SML-ROIK02-EX	1 kit
Detection of protein carbonyls in tissue and cell lysates by Western blotting Protein Carbonyls Western Blot Detection Kit (15 Blots)	SML-ROIK03-EX	1 kit
Detection of protein carbonyls by immunohistochemical staining. Protein Carbonyls Immunohistochemical Staining Kit (50 slides)	SML-ROIK04-EX	1 kit



COSMO BIO Co., LTD.

TOYO EKIMAE BLDG. 2-20, TOYO 2-CHOME,
KOTO-KU. TOKYO 135-0016, JAPAN
TEL : (81)3-5632-9617
FAX : (81)3-5632-9618
e-mail : export@cosmobio.co.jp
URL : www.cosmobio.com

