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Controls

Antibodies

Enzymes

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Specialty Chemicals

Bioxytech® SOD–525 Assay 21010

Quantitative Measurement of Superoxide Dismutase Enzyme Activity

For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 21010

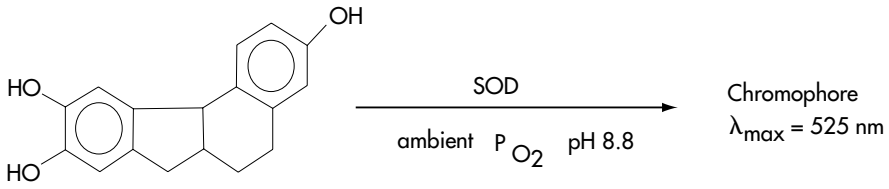
The Superoxide dismutases (SOD) are a family of metalloproteins. These enzymes catalyze the decomposition of the superoxide radical into hydrogen peroxide and oxygen.

$$2\ O_2^{\bullet-} + 2\ H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

SOD constitutes the primary defense against the toxic effect of superoxide in aerobic organisms. Currently, four isoforms of SOD have been identified; three (Cu/Zn-SOD, Mn-SOD and EC-SOD) in eukaryotes and one (Fe-SOD) in Prokaryotes. In mammals, two of three SOD isoforms have Cu and Zn in their catalytic center and are localized intracellularly (Cu/Zn-SOD or SOD-1) or extracellularly (EC-SOD or SOD 3). SOD-1 (M.W. 32 kDa) has been found in the cytoplasm, nuclear compartments and lysosomes in mammals. SOD 3 (heterodimer, M.W. 135 kDa with high affinity to heparin) is the most recently discovered and least characterized member of the SOD family. It was first detected in plasma, lymph, ascites and cerebrospinal fluids. The expression of SOD-3 is highly restricted to specific cell types and tissues where its activity can exceed that of SOD-1. Mn-SOD or SOD-2, the third form of mammalian SOD, has manganese as a cofactor and is localized to the mitochondria of aerobic cells.

SOD-525 Method

The Bioxytech SOD-525 Assay takes advantage of a proprietary reagent whose alkaline autoxidation is accelerated by the presence of SOD. The autoxidation product absorbs light at 525 nm. Rates of auto-oxidation are determined spectrophotometrically in the presence and absence of an SOD sample.



The ratio of the two rates measured can be converted directly to SOD concentration in the sample with the aid of a table (or equation) provided. No calibration is required however an SOD control (27617) is available for use with this kit.

U.S. Patent No. 5,543,298

References

1. Nebot C., Moutet M., Huet P., Xu J.Z., Yadan J. C., Chaudière J. Spectrophotometric assay of superoxide dismutase activity based on the activitated autoxidation of a tetracyclic catechol. Analytical Biochemistry 1993; 214:442-451.

2. Zelko, I .Et al. Superoxide Dismutase Multigene Family: A Comparison of CuZn-SOD (SOD1), Mn-SOD (SOD2), And EC-SOD (SOD-3) Gene Structures, Evolution, and Expression. 2002 FRBM 33:337-349

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SOD-525 Product Summary

Intended Use:	Quantitative measurement of superoxide dismutase activity. For Research Use Only. Not for Use in Diagnostic Procedures.
Format:	100 Test Colorimetric
Kit Contents:	• Chromogenic Reagent (R1) • Mercaptan Scavenger (R2) • Buffer
Storage and Stability:	18 months from date of manufacture when stored as specified.
Specimen Requirements:	Tissue and/or cell homogenates. (e.g. cell culture, WBC, RBC, etc.)

Assay Precision:

Replicates = 30	Level 1 Day1/Day2	Level 2 Day1/Day2	Level 3 Day1/Day2
Mean (U-525/mL)	0.82/0.81	2.85/2.86	13.60/13.22
Standard Dev	0.06/0.10	0.25/0.23	1.29/1.42
Total % CV	7.32/12.35	8.77/8.04	9.48/10.74

Assay Range:	Assay has best precision at Vs/Vc ratios less than 8.
Sensitivity:	LLD (SOD-525 U/mL) = 0.1 in the assay

SOD-525 Selected Research Applications

The Bioxytech SOD-525 assay has been used in research for measuring both SOD-1 and SOD-2 in a variety of models including melatonin pharmacology, cobalt and copper toxicity, preeclampsia related fetal hypoxia, diabetes, neurological diseases (amyotrophic lateral sclerosis disease or ALS), aging, gastrointestinal disorders, cystic fibrosis, pancreatic-fibrosis, asthma, organ transplantation and blood transfusion.

Perinatal encephalopathy induced by hypoxia or ischemia is a major cause of childhood neurological disability. Using OXIS' SOD-525 assay in a rat model, the Okatani group showed that the melatonin application to the mother increased antioxidant enzyme activities, including SOD, in the fetal brain and may thereby provide indirect protection against free radical injury (1). Ishi et al. showed that SOD activity is increased in the renal cortex in a streptozotocin induced diabetic rat model (2). In asthma patients, reduction of SOD activity has been associated with the severity of the disease (3). Improper antioxidant supplementation was shown to cause depletion of endogenous antioxidants (SOD, GSH and cGPx) and increased lipid peroxidation in cystic fibrosis patient blood (4). The Matsumura group studied the pancreatic injury in rats showing that SOD activity was significantly reduced in rats with pancreatic-fibrosis (5). In aging human populations, decreased SOD activity accompanied by increased lipid peroxidation has been reported (6). The Abe group showed that both "neo-red cells" (RBC substitutes) and RBC maintained the same SOD activity and other physical properties such as oxygen consumption and biocompatibility in an organ transplantation model. Thus, they suggested that "neo-red cells", which contain SOD, may be useful as an artificial oxygen carrier in blood transfusions and may reduce oxidative stress in organ transplantations (7).

1. Melatonin increases activities of glutathione peroxidase and superoxide dismutase in fetal rat brain Okatani, Y. et al. (2000) *J. Pineal Res.* 28: 89-96.
2. Nitric Oxide synthesis and oxidative stress in the renal cortex of rats with diabetes mellitus. Ishi, N et al. *J. Am. Soc. Nephrol.* 12:1630-39.
3. Lipid peroxidation as determined by plasma isoprostanes is related to disease severity in mild asthma. Wood LG et al. (2000). *Lipids* 35(9): 967-74.
4. Increased plasma fatty acid concentration after respiratory exacerbations are associated with elevated oxidative stress in cystic fibrosis. Wood LG et al. (2002). *Am. J. Clin. Nutr.* 75: 668-75.
5. Study of free radical and pancreatic fibrosis-pancreatic fibrosis induced by repeated injections superoxide dismutase inhibitor. Matsumura N et al. (2001). *Pancreas* 22 (1) 53-61.
6. Aging is associated with increased lipid peroxidation in human hearts, but not with mitochondrial respiratory chain enzyme defects. Mior, O. et.al (2000) *Cardiovasc Res.* 47: 624-31.
7. Superoxide generation from human polymorphonuclear leukocytes by liposome-encapsulated hemoglobin. Abe, H et al. (2002). *Artificial Cells, Blood Substitute and Immob. Biotechnology* 29 (4): 275-283

Bioxytech® GSH–400 Assay 21011

Quantitative Measurement of Glutathione

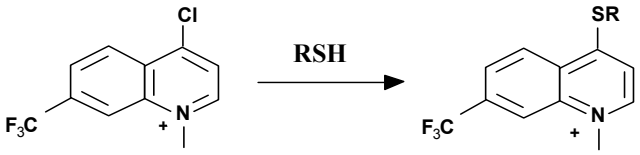
For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 21011

Glutathione (γ-glutamylcysteinylglycine or GSH) is a naturally occurring tripeptide whose nucleophilic and reducing properties play a central role in metabolic pathways, as well as in the antioxidant system of most aerobic cells. GSH plays a critical role as a coenzyme with a variety of enzymes including glutathione peroxidase, glutathione S-transferase, and thiol transferase. GSH also plays major roles in drug metabolism, calcium metabolism, γ-glutamyl cycle, blood platelet and membrane functions. In addition, GSH is crucial to a variety of life processes, including the detoxification of xenobiotics, maintenance of the -SH level of proteins, thiol-disulfide exchange, removal of hydroperoxides and free radicals as well as amino acid transport across membranes. Physiological values of intracellular GSH generally range from 1 to 10 mM. Although several methods have been described for the assay of GSH, reliable ones are often labor intensive and not easy to use. The GSH-400 from *Oxis*Research® provides a simple, specific test for GSH that does not require an enzyme as a reagent. Because of its simplicity, the GSH-400 Assay method is especially well adapted to the assay of glutathione in large series of biological samples.

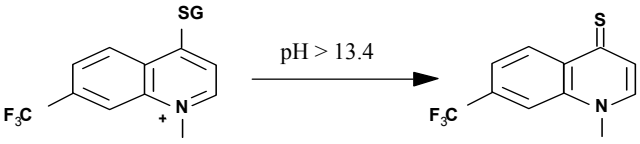
GSH-400 Method

The GSH-400 method is based on a chemical reaction that proceeds in two steps. The first step leads to the formation of substitution products (thioethers) between a patented reagent, R1 (4-chloro-1-methyl-7-trifluromethyl-quinolinium methylsulfate), and all mercaptans (RSH) present in the sample:



Reagent R1 **Thioethers**

The second step is a β-elimination reaction which takes place under alkaline conditions. The reaction is mediated by an R2 reagent (30% NaOH) that transforms the specific substitution product (thioether) obtained with GSH into a chromophoric thione having a maximal absorbance wavelength at 400 nm:



GSH-thioether **Thione**

The GSH-400 method makes it possible to specifically assay glutathione with only one sampling and one colorimetric measurement. A modification of this method can be used to assay other mercaptans. This is based on the measurement of thioethers, which absorb light at 356 nm in the absence of reagent R2.

Researchers are cautioned that although only "reduced" GSH reacts with R1 to form specific GSH-thioethers, some GSSG will become reduced *in vitro* by normal means causing some detection of GSSG in the assay. For exacting measurements of "total" GSH, OXIS recommends the GSH-420 (21023) kit or for "ratio" measurement, the GSH/GSSG-412 (21040) kit.

*U.S. Patent Number 5,817,520.

References

1. Blume K. G., Paniker N. V., Beutler E. (1975) Glutathione (Flohé L., Benöhr H. Ch., Sies H, Waller H. D., Wendel A. eds.), pp. 157–165, Academic Press, New York.

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21011

GSH-400 Product Summary

Intended Use:	Quantitative measurement of glutathione in biological samples. For Research Use Only. Not For Use in Diagnostic Procedures.
Format:	100 Test Colorimetric
Kit Contents:	• R1 Chromogenic Reagent • R2 (30% NAOH) • Buffer
Storage and Stability:	18 months from date of manufacture when stored as specified.
Specimen Requirements:	Tissue homogenates and cell lysates
Accuracy	With a series of 30 measurements performed on the same day and under the same experimental conditions, using GSH (20-200 µM), the standard error of the mean value (SEM) was less than 2%.
Reproducibility	With the same experiments performed twice at three-day intervals, using the same samples, the new SEM calculated on the two measurement series was lower than 2%.
Sensitivity:	5 µmol/L in the final reaction mixture

GSH-400 Selected Research Applications

The OXIS GSH-400 has been on the market for approximately 10 years and the citations supporting its use are numerous. Here we present a few examples of the versatility of this assay as regards its use in various sample types and conditions.

Baumer et al. evaluated effect of ROS on various parameters of male fertility. They studied the influence of ROS generated by a xanthine (X)-xanthine oxidase system on equine sperm motility, viability, acrosomal integrity, membrane potential and membrane lipid peroxidation (1).

Elsewhere, Demols et al. studied cerulein-induced acute pancreatitis (AP) and the effect on GSH by NAC in prophylactic (NAC given before the induction) and therapeutic groups (NAC given after the induction). The authors showed that NAC administration abolished the decrease in pancreatic and hepatic tissue GSH that normally occurs during AP (2).

Lonigro, R et al. used L-buthionine-(S,R)-sulfoimine (BSO) to irreversibly inhibit γ-glutamylcysteine synthetase and tested FRTL-5 cells for the effects of diminished GSH. The authors showed a direct correlation between intracellular GSH concentration and the expression of thyroid specific genes: thyroglobulin (TO) thyroid peroxides (TPO) which are mediated by the combination of TTF-1 (thyroid specific transcription factor), TTF-2 and Pax-8 (3).

Delcourt et al. evaluated GSH levels in erythrocytes of human subjects with cataracts showing that lower GSH levels correlate to increased incidence of cataracts (4).

Relating GSH content to immune function, Victor et al. performed an interesting timecourse study of GSH response to endotoxin mediated oxidative stress. They measured GSH levels in murine leuckocytes isolated from peritoneum, thymus, spleen and axillary nodes showing that the endotoxin LPS causes decreased GSH in slpeen and particularly axillary node immune cells.

1. The effect of reactive oxygen species on equine sperm motility, viability, acrosomal integrity, mitochondrial membrane potential, and membrane lipid peroxidation. Baumber J et al. (2000). J. Androl. 21: 895-902.

2. N-acetylcysteine (NAC) decreases severity of acute pancreatitis (AP) in mice. Demols A et al. (2000) Pancreas 20: 161-69.

3. Thyroid-specific gene expression is differentially influenced by intracellular glutathione level in FRTL-5 cells. Lonigro, R et al. (2000). Endocrinology 141: 901-909.

4. Risk factors for cortical, nuclear, and posterior sub-capsular cataracts.
Delcourt C et al. (2000). Am. J. Epidemiol. 151: 497-504.

5. Changes in the antioxidant content of mononuclear leukocytes from mice with endotoxiin-induced oxidative stress.Victor, VM et al. (2002). Mol. Cell Biochem. 229:107-111

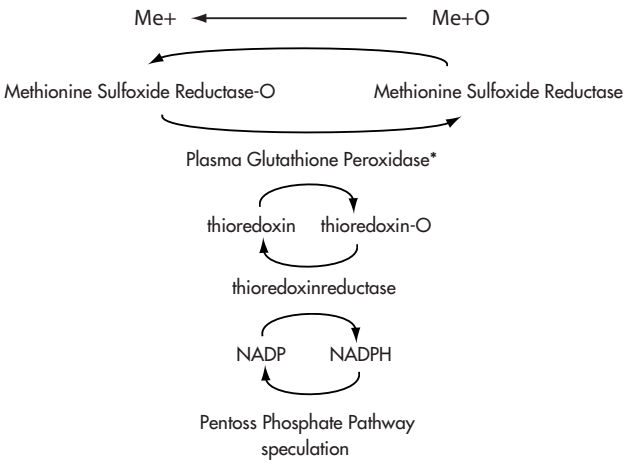
Bioxytech® Plasma GPx–EIA Assay 21014

Immunoassay for Quantification of Human Plasma Glutathione Peroxidase Enzyme Expression

For Research Use Only, Not Intended For Use in Diagnostic Procedures

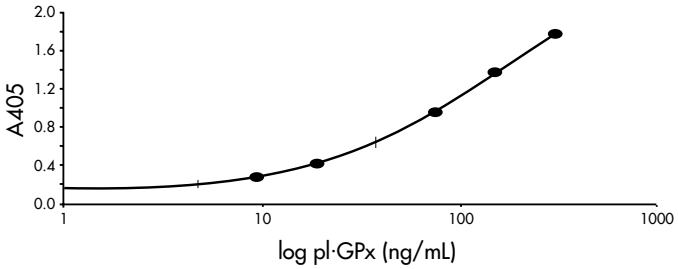
Catalog Number 21014

Glutathione peroxidases (GPx's) are selenoenzymes that catalyze the reduction of hydroperoxides (H_2O_2 or ROOH) in the presence of glutathione (GSH). Human blood contains, in addition to Se-GPx in erythrocytes, a plasma-specific glutathione peroxidase (pl•GPx). Glutathione peroxidase is a tetramer of approximately 94-100 kDA with each of the four identical subunits containing an active site with an essential selenocysteine residue. Plasma GPx differs from the other glutathione peroxidases by its primary sequence, its glycosylated N-terminal region, and its extra-cellular location. Initially purified from human plasma, pl•GPx has also been found in human milk. It has now been reported that pl•GPx is mostly synthesized and secreted by renal cells. pl•GPx may play a key role in the methionine sulfoxide reductase/thioredoxin system (see figure).



pl•GPx-EIA Method

The Standard curve is obtained by fitting the standard absorbance at 405 nm to the concentration of pl•GPx by 4-parameter logistic curve fit.



The Bioxytech pl•GPx-EIA assay system is a standard three (3) step "sandwich" ELISA. In step one, a plate bound (e.g. "solid phase") anti-human pl•GPx polyclonal antibody captures sample and/or standard pl•GPx. In step two, the bound pl•GPx antigen is detected using a biotin-labeled polyclonal anti-human pl•GPx. Finally, step three involves binding of a streptavidin/alkaline phosphatase conjugate to the biotinylated antibody. *p*-Nitrophenyl phosphate (pNPP) is then added causing the enzymatic hydrolysis of pNPP. The formation of yellow *p*-Nitrophenol is monitored at 405 nm and the amount of sample pl•GPx calculated against the color development of standards.

U.S. Patent Number US 5,861,262.

References

1. Maddipati, K.R. and Marnett, L.J., (1987) J. Biolog. Chem. 262:33, 17398–17403 Esworthy,
2. R.S., Arch. Biochem. Biophys. (1993) 307:1, 29–34.
3. OXIS unpublished results.

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21014

pl•GPx-EIA Product Summary

Intended Use:	Quantitative measurement of human plasma glutathione peroxidase. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	96 well ELISA
Kit Contents:	• 96 anti-pl•GPx Coated Microtiter Wells (6x16 strips) Plus Frame • Purified, Lyophilized pl•GPx Standard • Biotin Coupled Polyclonal Anti-pl•GPx • Streptavidin-Coupled Alkaline Phosphatase Solution • pNPP Tablets Plus Diluting Buffer • Stop Solution • Diluting and Wash Buffers
Storage and Stability:	12 months from date of manufacture when stored as specified.
Specimen Requirements:	Human serum or heparinized plasma.
Specificity:	No observed cross-reactivity with other GPx isoforms.
Sensitivity:	2.5 ng/mL

pl•GPx-EIA Selected Research Applications

Jorres et al. investigate the effect of repeated ozone exposure on inflammatory biomarkers. The OXIS plasma GPx-EIA is used to test for pl•GPx concentration in bronchioalveolar lavage fluid (BALF)

Delcourt et al studied the effect of sunlight exposure on human cataract pathogenesis.
Both physical and biological measurements (plasma glutathione peroxidase expression and superoxide dismutase activity, using the OXIS pl-GPX-EIA and SOD-525 assays respectively) were evaluated. Plasma was used for the pl•GPx-EIA and erythrocytes were tested for using the SOD-525 kit.

Wendland et al. studied plasma antioxidants, vitamins and lipid peroxidation among "healthy" patients and CD patients (smokers and malnourished subjects were eliminated from the study). Plasma GPx and plasma selenium concentrations were similar in both groups. They noted that previous reports, showing elevated plasma GPx in CD patients could be due to the involvement of smokers in those studies.

Atherosclerosis and cardiovascular complications are common features in end-stage renal failure patients (Glomerulonephritis, Renal hypoplasia, Polycystic kidney disease, Renal failure due to stasis and Bilateral nephrostomy). Donica et al. studied the relationships between lipid peroxidation products (MDA & 4-HNE) and plasma-GPx contents versus proinflammaotry cytokines (TNFa, IL-6 and soluble receptor, sIL-6R) contents in plasma samples. Plasma-GPx levels was lower in patients, compared to the control, but not significant.

1. The effect of repeated ozone exposures on inflammatory markers in brochoalveloar lavage (BAL) fluid and mucosal biopsies. Jorres, RA et al. (2000). Am. J. Respir. Crit. Care. Med. 161: 1855-61

2. Light Exposure and the risk of cortical, nuclear and posterior sub capsular cataracts.
Delcourt, C et al. (2000). Arch Ophthalmology 118(3): 385-392.

3. Lipid peroxidation and plasma antioxidant micronutrients in Crohn's disease.
Wendland, B.E., et. al., (2001) Am. J. Nutr. 74(2):259-264

4. Evaluation of lipids peroxidation products versus proinflammatory cytokines in hemodialized patients.
Donica, H. (2001) Renal Failure 23(2): 231-38

Bioxytech® cGPx–340 Assay 21017

Quantitative Measurement of Cellular Glutathione Peroxidase Enzyme Activity

For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 21017

Cellular glutathione peroxidase (cGPx) is a member of a selenium containing family of GPx enzymes whose function is to detoxify peroxides in the cell. Since peroxides decompose to form highly reactive radicals, the GPx enzymes play a critical role in protecting the cell from free radical damage, particularly lipid peroxidation. The GPx enzymes catalyze the reduction of H_2O_2 to water and organic peroxides (ROOH) to the corresponding stable alcohols (ROH) using glutathione (GSH) as a source of reducing equivalents.

$$ROOH + 2\ GSH \xrightarrow{GPx} ROH + GSSG + H_2O$$

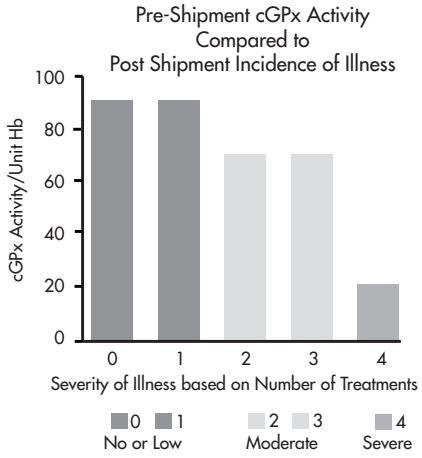
With few exceptions, GPx enzymes are comprised of 4 identical subunits. Each subunit contains a molecule of selenocysteine in the enzyme's active site. When associated with GSH, GPx selenocysteine is thought to participate directly in electron donation to the peroxide substrate and to become oxidized in the process. This oxidation of selenocysteine then strips electrons from 2 units of GSH generating, disulfide oxidized glutathione GSSG. Since cGPx activity depends on selenium the Bioxytech cGPx-340 assay is an ideal functional assay for detecting the effect of Se specific trace mineral deficiency.

cGPx-340 Method

The Bioxytech cGPx-340 method is an indirect measure of cGPx. Oxidized glutathione (GSSG), produced upon the reduction of organic peroxides by cGPx, is recycled to its reduced state by the enzyme glutathione reductase (GR).

$$GSSG + NADPH + H^+ \xrightarrow{GR} 2GSH + NADP^+$$

To assay for GPx activity, samples are added to a solution containing GSH, glutathione reductase (GR) and NADPH. An enzymatic reaction is initiated by addition of *tert*-butyl hydroperoxide substrate. By monitoring the NADPH consumption, as noted by a decrease in absorption at 340 nm, the Bioxytech cGPx-340 method allows for sensitive detection of cGPx activity. The graph below illustrates the usefulness of the Bioxytech assay as a functional enzyme biomarker. In this example, blood samples were taken from auction yard feeder calves and assayed for cGPx activity using the Bioxytech cGPx-340 test. After shipment to the feedyard, calves were monitored for signs and treatment of bovine respiratory disease complex (BRD). The figure below shows correlation between pre- shipment cGPx values and predisposition for BRD based on symptoms and incidence of treatment.



References

1. Paglia, D.E., Valentine, W.N., Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. July (1967) 70:158–169.

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cGPx-340 Product Summary

Intended Use:	Quantitative measurement of cellular glutathione peroxidase activity. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	100 test colorimetric
Kit Contents:	• NADPH Reagent *Contains NADPH, GSH and GR* • Assay Buffer • *tert*-Butyl Hydroperoxide
Storage and Stability:	21 months from date of manufacture when stored as specified.
Specimen Requirements:	Cell and tissue lysates

Assay Precision:	Low	Med	High
Inter- assay (% CV)	4.0	4.1	4.9

Sensitivity: 6 mU/mL *(final assay concentration)*

cGPx-340 Selected Research Applications

GPx-1 or cellular-GPx has been identified and studied in erythrocytes, lens, liver, brain, heart, platelet, lungs and other tissues. The cGPx-340 Assay has been used in research to study various conditions including ischemia/reperfusion injury, diabetes, neurological disorders, cystic fibrosis, cancer, preeclampsia, gastrointestinal, metal toxicity, and others.

Transplantation of any organ necessarily involves an unavoidable period of ischemia. In a research study using the OXIS cGPx-340 assay, Seth et al. showed that treatment with Picroliv (a natural antioxidant) protects rat renal tissues from ischemia-reperfusion injury by modulating antioxidant activities such as cGPx. (1). Janciauskiene et al. examined cGPx levels in relation to LDL receptor negative and LDL receptor positive cell lines in a study targeting the detrimental cardiovascular effect of amyloid fibril deposits (IAPP) in Type 2 diabetes. They showed that elevated cGPx levels correlated with decreased lipid peroxidation and hence minimizing IAPP formation (2). Using a rat model, Parikh et al. demonstrated that some antipsychotic drugs (e.g., atypical antipsychotics: Clozapine, Risperidone, and Olanzapine) reduced oxidative stress and oxidative cell injuries by increasing the levels of antioxidants such as GPx, catalase and Cu/Zn-SOD and decreasing lipid peroxidation compared to the typical drug treatment, Haloperidol (3). Cystic Fibrosis is caused by the loss or dysfunction of the CFTR/Cl^- channel. The resulting defective ion permeability leads to viscous epithelial secretion. In general, energy requirements of CF patients are 120-150% higher than the "normal" requirement, despite the administration of pancreatic enzyme supplement and others. However, it has been shown that a high fat diet without "proper" antioxidant supplementation may lead to higher degree of oxidation (e.g. lipid peroxidation) as well as endogenous antioxidant "depletion" such as GSH and cGPx (4). A research study in a murine model of somatic mutagenesis (cancer), using various OXIS kits (GPx-340, Catalase-520 and GSH/GSSG-412), the Felix group demonstrated that glucose-6-phosphate dehydrogenase (G6PD) is required for limiting oxidative mutageneisis in the mouse spleen (5). Preeclampsia is a leading cause of maternal and neonatal mortality and morbidity. Okatani et al. showed that melatonin could be useful in treating this condition and possible other clinical states involving excessive free radical production, such as intrauterine fetal growth retardation and fetal hypoxia (6). They showed GPx and SOD were significantly increased in chorionic homogenates from treated women, compared to the controls.

1. Prevention of renal ishemia-reperfusion-induced injury (IRI) in rats by Picroliv. Seth, PS et al. (2000) Biochem. Pharmacol. 59: 1315-22

2. Fibrillar islet amyloid polypeptide differentially affects oxidative mechanisms and lipoprotein uptake in correlation with cytotoxicity in two insulin-producing cell lines. Janciauskiene S and Bo Ahren (2000). Biochem. BioPhy. Res. Com. 267: 619-25.

3. Differential effects of antipsychotics on expression of antioxidant enzymes and membrane lipid peroxidation in rat brain. Parikh, V. et al.(2003) J. Psychiatric Research 37: 43-51

4. Increased plasma fatty acid concentration after respiratory exacerbations are associated with elevated oxidative stress in cystic fibrosis . Wood LG et al. (2002). Am. J. Clin. Nutr. 75: 668-75.

5. Redox imbalance and mutagenesis in spleens of mice harboring a hypomorphic allele of *Gpdx* ª encoding glucose 6-phosphate dehydrogenase. Felix, K et al.(2003) Free Radical Biology & Medicine 34 (2): 226-232.

6. Melatonin stimulates glutathione peroxide activity in human chorion. Okatani Y et al. (2001). J. Pineal Res. 30: 199-205

Bioxytech® GR–340 Assay 21018

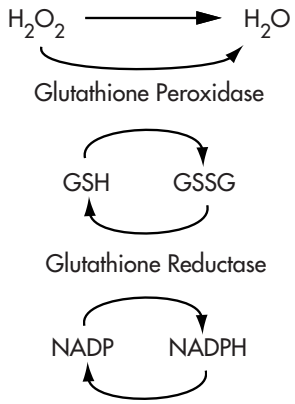
Quantitative Measurement of Glutathione Reductase Enzyme Activity

For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 21018

Glutathione reductase (GR, EC 1.6.4.2) is a ubiquitous enzyme, which catalyzes the reduction of oxidized glutathione (GSSG) to glutathione (GSH). Glutathione reductase is essential for the glutathione redox cycle that maintains adequate levels of reduced cellular GSH. GSH serves as an antioxidant, reacting with free radicals and organic peroxides, in amino acid transport, and as a substrate for the glutathione peroxidases and glutathione S-transferases in the detoxification of organic peroxides and metabolism of xenobiotics, respectively. This homodimeric enzyme is a member of the family of flavoprotein disulfide oxidoreductases. Each subunit has four domains; beginning at the N-terminus: an FAD-binding domain, an NADPH-binding domain, a central domain, and an interface domain. The active site of GR is at the dimeric interface. Since the GSSG binding site is composed of residues from both subunits, only the dimeric form is active.

Oxidized glutathione is reduced by a multi-step reaction in which GR is initially reduced by NADPH forming a semi-quinone of FAD, a sulfur radical and a thiol. The reduced GR (GR_{red}) reacts with a molecule of GSSG, resulting in a disulfide interchange, which produces a molecule of GSH and the GR_{red}-SG complex. An electron rearrangement in GR_{red}-SG results in a second disulfide interchange, splitting off the second molecule of GSH and restoring the GR to the oxidized form.



GR-340 Method

The GR-340 assay is based on the oxidation of NADPH to $NADP^+$ catalyzed by a limiting concentration of glutathione reductase.

$$GSSG + NADPH + H^+ \xrightarrow{\text{Glutathione Reductase}} 2\ GSH + NADP^+$$

One GR activity unit is defined as the amount of enzyme catalyzing the reduction of one micromole of GSSG per minute at pH 7.6 and 25°C. As shown in the above reaction, one molecule of NADPH is consumed for each molecule of GSSG reduced. Therefore, the reduction of GSSG is determined indirectly by the measurement of the consumption of NADPH, as demonstrated by a decrease in absorbance at 340 nm (A340) as a function of time.

References

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2. Massey V, Williams CH, J Biol Chem (1965) 240(11), 4470–4480.
3. Worthington DJ, Rosemeyer MA, Eur J Biochem (1976) 67, 231–238.
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GR-340 Product Summary

Intended Use:	Quantitative measurement of Glutathione Reductase activity. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	100 test colorimetric
Kit Contents:	• NADPH • Oxidized Glutathione (GSSG) in Potassium Phosphate Buffer. • Potassium Phospate Buffer ($K•PO_4$) • Diluent Buffer
Storage and Stability:	18 months from date of manufacture when stored as specified.
Specimen Requirements:	Tissue homogenates and/or cell lysates.

Assay Precision:

n=20	Low	Med	High
Mean mU/mL	2.36	4.60	9.12
SD Total	0.22	0.40	0.72
Total (%CV)	9.20	8.60	7.80

Assay Range:	Minimum Net Rate = 0.0050 A_{340}/Min (10x typical blank rate) Maximum Net Rate = 0.0625 A_{340}/Min Corresponding to 0.8-10.0 mU SOD-525
Sensitivity:	0.14 mU/mL (final concentration in the assay)

GR-340 Selected Research Applications

The GR-340 assay has been used for many purposes including the study of emphysema related functional muscle impairment, Cystic Fibrosis related research and various liver toxicology studies.

Skeletal muscle functional impairment has been reported in emphysema (EMP) and COPD patients. Mattson et al. looked at markers of oxidative stress in skeletal muscles in a porcine elastase induced hamster model of emphysema. They showed that EMP may induce oxidative stress in peripheral skeletal muscle. The study reported elevated GR activities in gastronemus muscle of hamsters with emphysema compared to controls (1). Velsor et al studied the levels of endogenous antioxidants in Wild type vs CFTR knockout mice. They reported elevated levels of GR activity as measured with the OXIS GR-340 kit (2). Christova et al. looked at the effect of Cobalt induced oxidative stress on liver antioxidant enzymes. They showed that GR activities increase along with GSH and heme-oxygenase-1 under chronic cobalt exposure conditions (3). In a separate publication by the same author, acute effects of cobalt administration were compared in rat and guinea pig. Again they showed significant remodeling of the antioxidant biomarkers, including GR, as a result of acute cobalt induced oxidative stress.

1. Lipid peroxidation in the skeletal muscle of hamsters with emphysema.
Mattson, J.P. et. al., (2002) Pathopysiology 8:215-221.

2. Antioxidant imbalance in the lungs of cystic fibrosis transmembrane conductance regulator protein mutant mice. Velsor et al. (2001) Am. J. Physiol. Lung Cell Mol. Physiol. 281:L31-38.

3. Heme oxygenase (HO) is the main protective enzyme in rat liver upon 6 day administration of cobalt chloride. Christova et al. (2001) Arch. Toxicology 75: 445-451.

4. Enhanced heme oxygenase (HO) activity increases the antioxidant defense capacity of guinea pig liver upon acute cobalt chloride loading: comparison with rat liver. Christova et al. (2002) Comparative Biochem, & Physiol. Part C 131:177-184.

Bioxytech® GSH-420 Assay 21023

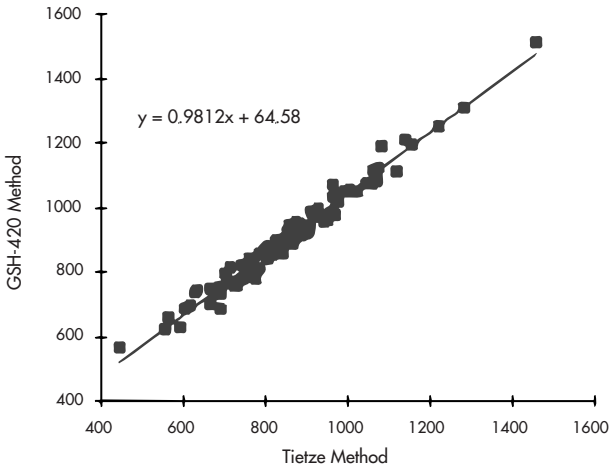
Quantitative Measurement of Total Glutathione

For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 21023

Reduced glutathione is a ubiquitous tripeptide (γ-glutamyl-cysteinylglycine) which functions as a coenzyme in amino acid transport, detoxification of xenobiotics and carcinogens, synthesis of DNA precursors and as an antioxidant. The Bioxytech®, GSH-420 is the next generation of the popular GSH-400 assay for the quantitative, colorimetric determination of glutathione in biological samples. This new assay allows for measurement of "total" GSH.

The GSH-420 compares favorably with other commercially available assay methods. The figure to the right shows the correlation between the GSH-420 and the DTNB-enzyme (Glutathione Reductase) recycling assay which is used in the GSH/GSSG-412 assay from *Oxis*Research®. The correlation coefficient obtained by linear regression is 0.9874.



GSH-420 Method

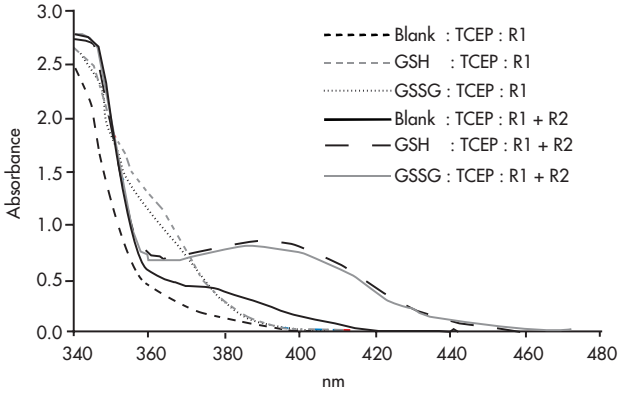
This assay is based on the formation of a chromophoric thione. There are three major steps to the reaction:
1. Sample is buffered and the reducing reagent tris (2-carboxyethyl) phosphine (TCEP) is added to reduce any oxidized glutathione (GSSG) present in the sample.
2. The chromagen, 4-chloro-1-methyl-7-trifluoromethyl-quinolinium methylsulfate, is added to the sample forming thioethers with all thiols.
3. The pH is raised by base addition resulting in a β-elimination reaction and formation of a chromophoric thione specific to GSH.

The measurement is made at 420 nm to negate the contribution by the thioether pool having peak absorbance at 356 nm. The figure below shows characteristic spectra generated after addition of base.

Improvements over existing GSH-400 assay:
- No ambiguity in "total" vs. "reduced" measurement.
- Greater specificity
- Precipitation reagent included

** Note: Omission of reducing agent allows for approximation of reduced GSH as with the existing GSH-400 assay.*

*U.S. Patent Number 5,817,520



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21023

GSH-420 Product Summary

Intended Use:	Quantitative measurement of "total" glutathione. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	100 test colorimetric
Kit Contents:	• Assay Buffer • Chromogen • Color Developer • Reducing Reagent • Precipitation Reagent • Calibrators (low and high)
Storage and Stability:	21 months from date of manufacture when stored in the dark at 2-4°C.
Specimen Requirements:	Erythrocyte lysates, whole blood, tissue homogenates

Precision:	Low	High	Blood
Mean	124.3	248.6	687.7
Intra-assay (% CV)	2.3	1.6	2.6
Inter-assay (% CV)	5.8	3.5	5.8
Total Precision (% CV)	6.0	3.7	6.1

Sensitivity: 9 µM in the sample

GSH-420 Selected Research Applications

The GSH-420 has not been on the market as long as GSH-400 however has already been used in a wide variety of studies.

In an animal feed supplement study, Williams et al. evaluated the safety and antioxidant potential of Lipoic acid (LA), a water-soluble and fat-soluble antioxidant, in horses undergoing light voluntary pasture exercise. They tested for endogenous antioxidant status (i.e. GSH and cGPx) using the GSH-420 and cGPx-340 kits on the automated OxyScan™ Analyzer. Lipid hydroperoxides were also evaluated using the OXIS LPO-560 Assay. They showed that LA supplemenation of 10 mg/kg did not result in any adverse signs/effects over a 14-day period and moderately reduced the oxidative stress of horses that were allowed light activity (1).

In a performance animal related study Hargreaves et al. tested the antioxidant status in horses during prolonged and strenuous endurance exercise (2). They showed both erythrocyte GSH and Vitamin C were decreased during exercise. Similarly cellular GPx concentration tested with the OXIS GPx-340 assay was 4-fold higher before the exercise. They showed that changes of antioxidant status such as cellular enzymatic antioxidants (e.g. cGPx) are good indicators of muscle leakage associated with muscle damages and hypo-dehyration.

In one of the more innovative uses of our GSH-420 assay Downs et al. studied oxdative stress in corals and showed that coral (Zooxanthelee) bleaching may be a final strategy to defend coral from oxidative stress (3). They showed high levels of antioxdant enzymes and heat shock proteins were negatively correlated with levels of oxdiative damage products.

1. Lipoic acid as an antioxidant in mature thoroughbred geldings: A preliminary study.
Williams, C.A. et. al., (2002). J. Nutr. 132: 1628S-1631S

2. Antioxidant status of horses during two 80-km endurance races.
Hargraves, B.J. et al., (2002) J. Nutr. 132:1781S-1783S

3. Oxidative stress and seasonal coral bleaching.
Downs, CA et al,. (2002). Free Radic. Biol. Med. 33(4):533-543

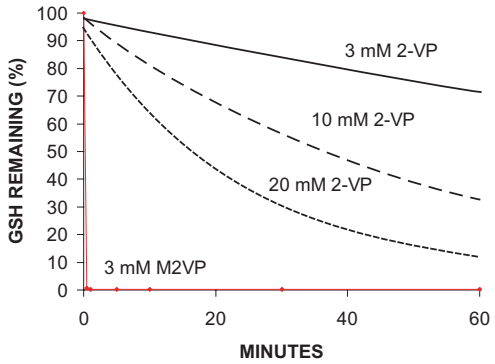
Bioxytech® GSH/GSSG-412 Assay 21040

Quantitative Measurement of Total Oxidized Glutathione Ratio

For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 21040

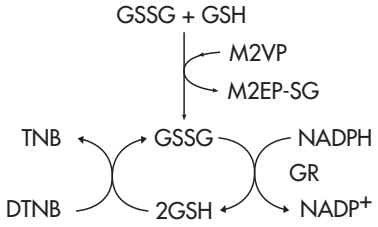
*Oxis*Research® has developed an improved method to measure the ratio of reduced to oxidized glutathione (GSH/ GSSG). This method features a more efficient mercaptan scavenger, 1-methyl-2-vinyl-pyridinium trifluoromethane-sulfonate (M2VP), to trap GSH. The figure below compares the scavenging abilities of the commonly used 2-vinylpyridine (2VP) with the OXIS M2VP reagent. One can easily see that M2VP is many times more effective in removing reduced GSH (99% within 1 minute) when compared to the 2VP reagent (11.7% within 1 minute).



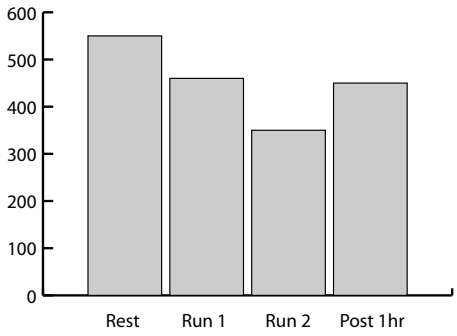
The increased scavenging capability of the OXIS M2VP reagent allows for more accurate measurement of small amounts of GSSG without overestimation.

GSH/GSSG-412 Method

The test method is based on the DTNB-GSSG reductase recycling assay for GSH first described by Tietze in 1969. The method employs glutathione reductase to ensure all GSH is in the reduced form capable of reacting with DTNB (also called Ellman's reagent) to form a spectrophotometrically detectable product at 412 nm.



The data below represents a study that was done using the OXIS GSH/GSSG-412 assay. During a segmented charity run, whole blood was sampled from healthy OXIS volunteers. Blood was collected, with and without the OXIS M2VP GSH scavenging reagent, and tested with the GSH/GSSG-412 assay. Note the decreasing ratio as subject undergoes progressively more strenuous exercise. Also note the ratio recovery after a 1 hour rest period. This data represents the utility of the GSH/GSSG-412 assay in effectively assessing oxidative stress relative to the glutathione system.



The GSH/GSSG-412 assay system contains reagents suitable for 100 tests representing the ratio of total glutathione to the oxidized form or 200 tests of either.

US Patent 5,543,298.

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2. Guntherberg H and Rost J (1966) Analytical Biochemistry 15, 205-210.
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21040

Intended Use:	Quantitative measurement of total gluthathione (GSH plus GSSG) and/or oxidized glu tathione (GSSG) alone. This assay allows for determination of the GSH/GSSG ratio. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	100 test spectrophotometric cuvette (ratio) or 200 test spectrophotometric cuvette (GSH or GSSG)
Kit Contents:	• Assay Buffer • GSSG Buffer • Enzyme • NADPH • Scavenger • Chromogen • Standards
Storage and Stability:	12 months from date of manufacture when stored as specified
Specimen Requirements:	Whole blood or tissue samples

Precision:

	GSSG in Buffer		Whole blood	
n=26	High	Low	GSH	GSSG
MeanRate at A_{412}	0.4194	0.0427	0.2938	0.00490
Intra-assay (% CV)	0.59	3.50	0.96	6.45
Inter-assay (% CV)	2.84	2.96	3.11	7.61
Total Precision (% CV)	2.87	3.86	3.18	8.86

Sensitivity: LLD in uM reaction mixture = 0.009
LLD in uM original sample = 0.54

GSH/GSSH-412 Selected Research Applications

Our GSH assays have been and continue to be used in various research model systems including pulmonary diseases, alcoholism, fertility and cancer models. Using the GSH/GSSG-412 kit, Cavarra E et al. studied cigarette smoking associated oxidative stress on human protease enzymes (specifically anti-trypsin activity) in a mouse model of chronic obstructive pulmonary disease (COPD). They demonstrated that acute smoke exposure *in-vivo* can result in significant changes to the antioxidant capacity of bronchoalveolar lavage fluid (BAL). These changes were ascribed to depletion of many factors including the perturbation in the glutathione redox system as revealed by the increase in oxidized GSH (GSSG).

In an alcoholism and fertility study, the Emaluelle group showed that when male rats were exposed to EtOH (8 wks of ethanol containing diet), there was increased paternal testicular oxidative injury demonstrated by decreased ratio of GSH/GSSG as well as enhanced lipid peroxidation (2).

In a diabetic model, Liang reported that metalothioneine-tranfected diabetic mice exhibit significantly lower levels of GSSG compared to the non-transfected diabetic mouse and a similar to "normal"/control mouse (3). Many diabetic patients suffer from cardiomyopathy, even in the absence of vascular disease. Therefore, these results demonstrate that cardiomyocyte-specific expression of an antioxidant protein may reduce the damage to diabetic heart.

Most recently, researchers at the National Cancer Institute (NCI), National Institutes of Health (NIH) and a German group studied the effects of G6PD activity in terms of general metabolism, regulation of oxidative stress and cancer (somatic mutagenesis) in mice. They showed that the level of "total GSH" did not change significantly in the brain tissues of G6PD deficient ($G6PD^-$) mice, compared to the "wild type". However, the GSH ratio (GSH/GSSG) was significantly increased (2-3 fold) in the "deficient" mice, as was their mutation rates ($G6PD^-$ = 24 – 30 $x10^{-5}$ vs. $G6PD^+$ = 6.95 ± 1.85 $x10^{-5}$) (4). The mutagenesis was measured by examining the mutant frequency in Lac-Z gene, the target reporter gene of mutagenesis in the shuttle vector, pUR288.

1. Human SLPI (secretory leukoprotease inhibitor) inactivation after cigarette smoke exposure in new in vivo model of pulmonary oxidative stress.
Cavarra E.. et al. (2001). *Am. J. Physiol Lung Cell Mol. Physiol* 282: L412-L417.
2. Peripubertal paternal EtOH exposure: testicular oxidative injury, fertility and offspring.
Emaluelle NV et al., (2001) *Endocrine* 14: 213-19.
3. Over expression of metallothionein reduces diabetic cardiomyopathy.
Liang Q et al. (2002) *Diabetes* 51 (1): 174-81.
4. Moderate G6PD deficiency increase mutation rates in the brain of mice.
Felix, K et al. (2002) *Free Radical Biology & Medicine* 32 (7): 663-73

Bioxytech® Catalase-520 Assay 21042

Quantitative Measurement of Catalase Enzyme Activity

For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 21042

Catalase is a ubiquitous antioxidant enzyme that is present in most aerobic cells. It serves as one of the body's defense systems against H_2O_2, a strong oxidant that can cause intracellular damage. It is found in high concentration in erythrocytes and liver while lower concentrations are found in skeletal muscle, brain and heart. Measurement of catalase activity can be useful as a research tool for certain diseases such as acute pancreatitis and some liver diseases where values are elevated. Each unit of catalase decomposes 1 μM of H_2O_2 per minute at 25°C and pH 7.0. The common method for the determination of catalase involves measuring the change in absorbance at 240 nm (hydrogen peroxide decomposition due to catalase activity) in one minute. Criticisms of the method include interference from non-specific UV absorbing materials and the requirement of quartz cuvettes. Because of this, *Oxis*Research® has developed a colorimetric endpoint assay. The release of the new Catalase-520 assay adds to the popular Bioxytech® family of Antioxidant Biomarkers and provides another useful tool for oxidative stress investigations.

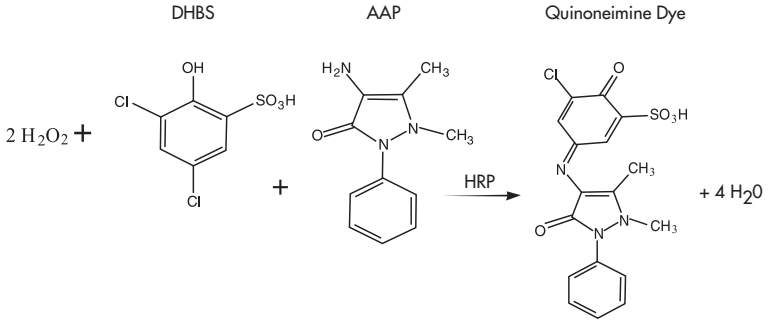
Catalase-520 Method

The *Oxis*Research® Catalase-520 assay employs a two-step reaction scheme.

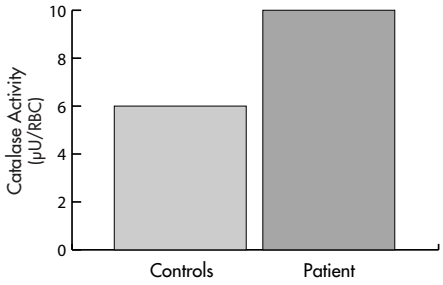
Reaction 1: Sample catalase reacts with a known quantity of H_2O_2. The reaction is stopped after 1 minute with sodium azide (NaN_3).

$$2\,H_2O_2 \xrightarrow{\text{catalase}} 2\,H_2O + O_2$$

Reaction 2: In the presence of horseradish peroxidase (HRP), remaining H_2O_2 reacts with 3,5-Dichloro-2-hydroxybenzenesulfonic (DHBS) acid and 4-aminophenazone (AAP) to form a chromophore with a color intensity inversely proportional to the amount of catalase in the original sample.



As a test of the assay's performance, Oxis compared red blood cell (RBC) catalase activity in a Thalassemia patient with that of a control population. In agreement with published values, the graph below shows increased activity in the patient relative to controls.



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3. Kirkman, H.N., et al., (1987) The Function of Catalase-bound NADPH, J. Biol. Chem. 262, 660-666.
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Catalase-520 Product Summary

Intended Use: Quantitative measurement of catalase enzyme activity. For Research Use Only. Not Intended For Use in Diagnostic Procedures.

Format: 100 test colorimetric

Kit Contents:
- Assay buffer
- Substrate
- Standards
- Sample Diluent
- Chromagen
- HRP
- Stop Reagent

Storage and Stability: 12 months from date of manufacture when stored as specified.

Specimen Requirements: Whole blood, serum, plasma, cell lysate and tissue

Assay Precision:

n=20	Low	High	Cell Lysate
Mean (U/mL)	14.1	56.7	38.7
Intra-assay (%CV)	2.39	0.78	1.29
Inter-assay (%CV)	4.03	2.13	5.55
Total Precision (%CV)	4.53	2.23	5.66

Sensitivity: LLD in reaction mixture = 0.097 U/mL
LLD in sample = 1.71 U/mL

Catalase-520 Selected Research Applications

The OXIS Catalase-520 assay is relatively new but we are already beginning to collect information regarding its use in current research models. Here we list two such citations wherein the kit was used to monitor catalase activity in relation to NADPH availability and G6PD activity then in a second paper as related to anti-psychotic drug treatment.

Felix et al showed that catalase activity in the murine spleen is diminished in G6PD knockout mice as compared to controls (1). This correlates with catalase's dependancy on NADPH to remain in the active state. Parikh et al studied endogenous antioxidant activities in the brain of Rats treated with various antipsychotic drugs and determined that chronic Haloperidol treatment had the effect of reducing Catalase, SOD and GPx axtivities as compared to animals treated with atypical antipsychotic drugs such as Clozapine, Risperidone and Olanzapine.

1. Redox imbalance and mutagenesis in spleens of mice harboring a hypomorphic allele of *Gpdx*^{a} encoding glucose 6-phosphate dehydrogenase. Felix, K. et al. (2003) Free Radical Biology and Medicine 34 (2): 226-232.

2. Differential effects of antipsychotics on expression of antioxidant enzymes and membrane lipid peroxidation in rat brain. Parikh, V. et al. (2003) J. Psychiatric Research 37:43-51

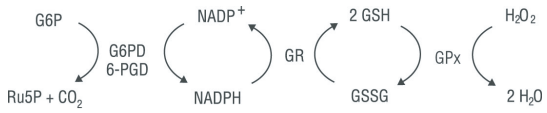
Bioxytech® G6PD¦6PGD-340 Assay 21045

Quantitative Measurement of Glucose-6-phosphate Dehydrogenase (G6PD) and 6-Phosphogluconate Dehydrogenase (6-PGD) Enzyme Activity

For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 21045

Since the amount of glutathione (GSH) present in the cell is finite, oxidized glutathione must be recycled to the reduced state to maintain protection against hydrogen peroxide. In cells experiencing oxidative stress, a continual supply of reducing equivalents, in the form of NADPH, is required in order to convert GSSG into GSH. The two reactions in the pentose phosphate pathway that produce NADPH are catalyzed by the enzymes glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6-PGD).

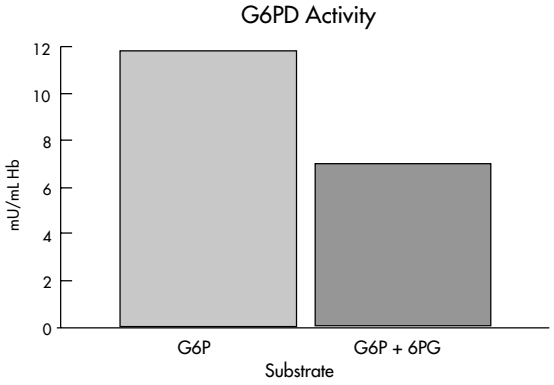


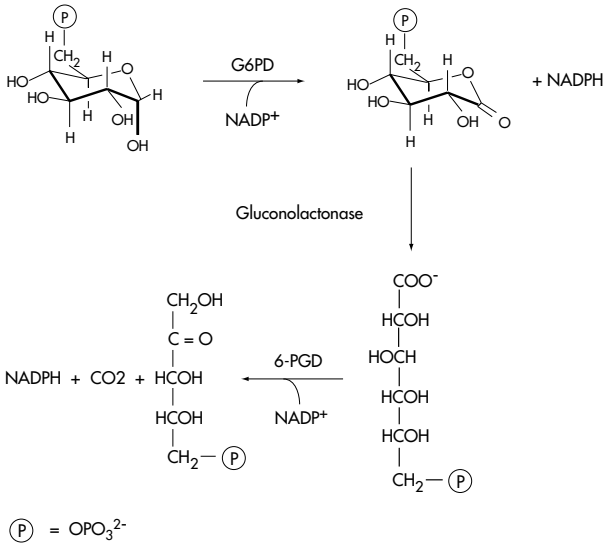
Of these two enzymes, G6PD is the predominant player in NADPH regulation and can play an important role in an organism's overall redox status and subsequent ability to handle oxidative stress. The easiest method for measuring G6PD activity is to monitor the production of NADPH in the presence of the appropriate substrate. A caveat of this method is that a product of the G6PD reaction produces substrate for the 6PGD reaction causing overestimation of G6PD when both G6PD and 6-PGD are present. In their failure to account for this contribution, other commercially available assays lack the specificity and subsequent sensitivity for studies relating G6PD activity and NADPH availability to oxidative stress. Because the G6PD¦6PGD-340 assay from *Oxis*Research® does discriminate between the two enzymes, even subtle changes in G6PD activity can be detected.

G6PD¦6PGD-340 Method

To avoid overestimation of G6PD and to obtain accurate measurement of both G6PD and 6-PGD activity, the OXIS G6PD 6PGD™ assay employs the method of Glock and McLean wherein two measurements of NADPH production are made: one with saturating concentrations of both glucose-6-phophate (G6P) and 6-phosphogluconic acid (6-PGA) and one with only 6-PGA.

G6PD activity is then calculated as the difference in rate of NADPH production between the assays with both substrates and only 6-PGA. The figure below illustrates the over-estimation of red cell G6PD when both substrates are not used.





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21045

G6PD:6PGD-340 Product Summary

Intended Use: Quantitative measurement of G6PD and/or 6PGD enzyme activity. For Research Use Only. Not Intended For Use in Diagnostic Procedures.

Format: 100 test colorimetric (single enzyme)
50 test colorimetric (both enzymes)

Kit Contents:
- Assay Buffer
- NADP Reagent
- G6P Substrate
- 6-PGA Substrate
- Sample Diluent

Storage and Stability: 12 months from date of manufacture when stored as specified

Specimen Requirements: Tissue, cell or RBC lysate

Precision:

	G6PD			6-PGD		
	Low	Med	High	Low	Med	High
mU/mL enzyme	2.46	4.54	8.50	3.08	5.40	9.00
Mean (ΔA_{340}/min)	0.0136	0.0403	0.0787	0.0192	0.0336	0.0560
Intra-assay (%CV)	2.07	1.14	2.45	1.65	1.38	1.30
Inter-assay (%CV)	1.62	1.05	2.42	1.84	1.25	1.42

Sensitivity:

	G6PD	6-PGD
LLD in reaction mixture (mU/mL)	0.024	0.027
LLD in sample (mU/mL)	0.48	0.54

Bioxytech® GST-340 Assay 21046

Quantitative Measurement of Glutathione S-tranferase Enzyme Activity

For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 21046

Glutathione S-transferase (GST) utilizes glutathione (GSH) to scavenge electrophilic xenobiotics as part of an organism's defense mechanism against the mutagenic, carcinogenic and toxic effects of such compounds. GST activity is present throughout the phylogenetic scale, generally as a family of enzymes whose major classes differ in their xenobiotic specificity. Each major class contains multiple isozymes. All GST isozymes catalyze the same reaction, shown schematically below, where X is a xenobiotic.

$$X + GSH \xrightarrow{GST} G\text{-}S\text{-}X + H^+$$

GST activity is present in most human tissues but the expression of the different isozymes is organ specific. It is especially prevalent in the liver, which plays a major role in detoxification. Due to its activity in detoxifying xenobiotics, GST plays an important role in the well-being of all living systems. Because of the interaction of GST with glutathione, a major component of the antioxidant system, and because of the implications relative to removal of oxidatively generated harmful substances, *Oxis*Research® has introduced the GST-340 assay for measurement of GST activity.

Because the various GST isozymes have widely varying Km and Vmax values towards different xenobiotics and GSH, the contribution of each to the overall GST activity will depend on the xenobiotic used as well as the reaction conditions. For this reason the substrate with the broadest range of isozyme detectability coupled with ease of monitoring was chosen for the GST-340 activity assay.

GST-340 Method

The Bioxytech GST-340™ assay is based on the GST-catalyzed reaction between 1-chloro-2,4-dinitrobenzene (CDNB) and GSH. Following addition of GST to the reaction vessel CDNB and GSH combine to form a dinitrophenyl thioether chromophore and a chloride ion (Figure 1).

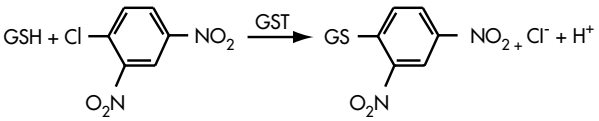

Figure 1 - The Reaction of CDNB with GSH

One unit of GST activity is defined as the amount of enzyme producing 1 μmol of CDNB-GSH conjugate/min under the conditions of the assay. Figure 2 shows how addition of GST results in the gradual increase of the chromophore. The non-enzymatic reaction of CDNB and GSH under assay conditions is slow relative to the enzyme-catalyzed reaction.

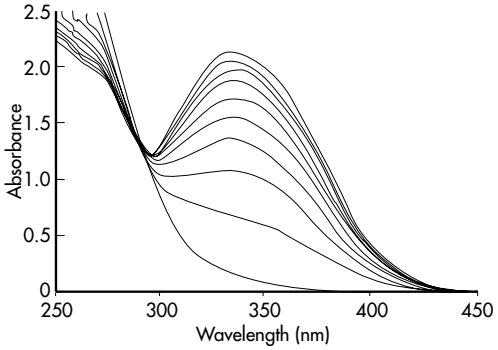

Figure 2. (right) The reaction of CDNB and GSH in the presence of GST. The lowest scan at 340 nm is the CDNB/GSH mixture before addition of GST. Scans were taken at two minute intervals.

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3. Landi, S. (2000) Mammalian Class Theta GST and Differential Susceptibility to Carcinogens: A Review, Mutation Res. 463, 247-283.
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21046

GST-340 Product Summary

Intended Use:	Quantitative measurement of glutathione S-tranferase enzyme activity. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	100 test colorimetric
Specimen Requirements:	Tissue, cell or RBC lysate
Storage and Stability:	9 months from date of manufacture when stored as specified
Kit Contents:	• Assay Buffer • CDNB Substrate • GSH • Sample Diluent

Precision:	Low	Med	High
mU/mL enzyme	1.42	4.20	8.20
Mean (ΔA_{340}/min)	0.0136	0.0403	0.0787
Intra-assay (%CV)	0.89	0.80	1.95
Inter-assay (%CV)	1.05	0.89	2.13

Sensitivity:	
LLD in reaction mixture (mU/mL)	0.060
LLD in sample (mU/mL)	1.2

Bioxytech® AOP-490 Assay 21052

Quantitative Measurement of Antioxidant Potential in Biological Samples

For Research Use Only, Not Intended For Use in Diagnostic Procedures

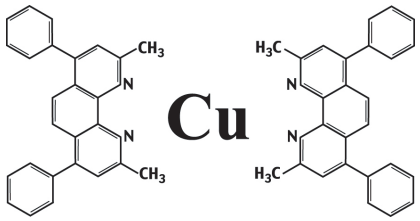
Catalog Number 21052

Four antioxidant strategies can be found in biological systems: (1) enzymatic (superoxide dismutase, glutathione peroxidase and catalase), (2) large molecules (albumin, ceruloplasmin and ferritin), (3) small molecules (ascorbic acid, a-tocopherol, β-carotene, plasma ubiquinol, uric acid, and glutathione) and (4) some hormones (estrogen, melatonin, angiotensin and others). These antioxidants can be found either as water-soluble or fat-soluble molecules. *Oxis*Research® offers many assays for detection of specific, endogenously expressed enzymes and now offers the AOP-490 assay for detection of overall, antioxidant potential in aqueous biological samples.

This new assay compares well to the popular ORAC (Oxygen Radical Absorbance Capacity) assay without the need for fluorescence detection and requires only 3 minutes to perform compared to the 1 hour plus timeframe for ORAC. The AOP-490 assay also offers advantages over other existing assays such as FRAP (Ferric Reducing Ability) and TEAC (Trolox Equivalent Antioxidant Capacity) due to its ability to measure all antioxidant contributions (including thiol containing compounds) with little interference due to endogenous Reactive Oxygen Species (ROS).

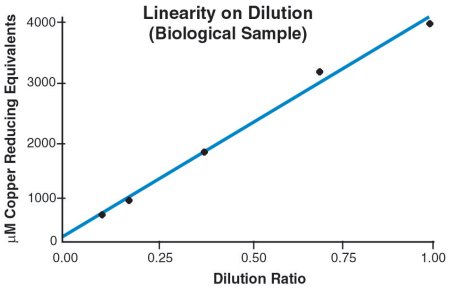
AOP-490 Method

The Bioxytech® AOP-490 Assay is based upon the reduction of Cu^{++} to Cu^{+} by the combined action of all antioxidants present in the sample. A chromogenic reagent, bathocuproine (2,9-dimethyl-4, 7-diphenyl-1, 10-phenanthroline), selectively forms a 2:1 complex with Cu^{+}. The resulting chromophore, having a maximal absorbance at 490 nm, provides the basis for quantification of antioxidant potential.



Using the standard provided, results of the assay may be expressed either as "μM Uric Acid Equivalents" directly from the plot of absorbance change versus mM uric acid concentration, or as "μM Copper Reducing Equivalents," using a given conversion factor.

The Bioxytech® AOP-490 is capable of measuring of antioxidant potential in biological samples across a wide range of dilutions with excellent recovery.



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21052

AOP-490 Product Summary

Intended Use:	Quantitative measurement of antioxidant potential in biological samples. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	100 test colorimetric
Kit Contents:	• R1 (containing bathocuproine) • R2 (containing Cu^{++}) • Uric Acid standard • Stop solution
Storage and Stability:	12 months from date of manufacture when stored as specified.
Specimen Requirements:	Serum, plasma, tissues and other biological samples
Assay Precision:	
Intra-assay (%CV)	2.22
Inter-assay (%CV)	4.20
Sensitivity:	30 µM uric acid equivalents

Bioxytech® LPO–586 Assay 21012

Quantitative Measurement of Malondialdehyde (MDA) Plus 4-hydroxyalkenals (4HAE)

For Research Use Only, Not Intended For Use in Diagnostic Procedures

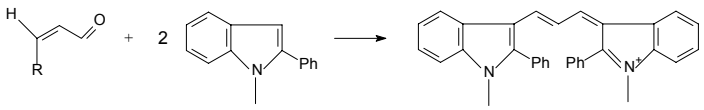
Catalog Number 21012

Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides are unstable and decompose to form a complex series of compounds including reactive carbonyl compounds. Polyunsaturated fatty acid peroxides generate malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) upon decomposition. These substances in tandem provide a simple biomarker for measurement of general lipid peroxidation. The LPO-586 method is best suited for measuring MDA in combination with 4-hydroxyalkenals although the kit provides reagents for specific measurement of MDA alone as well. Researchers are advised that it is not possible to differentiate HAE from MDA using this kit due to slight differences in reaction stoichiometry when comparing the hydrochloric acid (HCl) reaction with the methanesulfonic acid (MSA) reaction.

We recommend this kit for detection of MDA plus HAE as a general indication of lipid peroxidation in tissue and cell samples. For MDA only we recommend the MDA-586 assay (21044) or for HAE only, we recommend the HAE-586 assay (21043). For measurement of lipid peroxidation in plasma we recommend researchers look at lipid hydroperoxides (LOOH) using the OXIS LPO-560 assay or MDA using the OXIS MDA-586 assay coupled with 3rd derivative spectroscopy.

LPO-586 Method

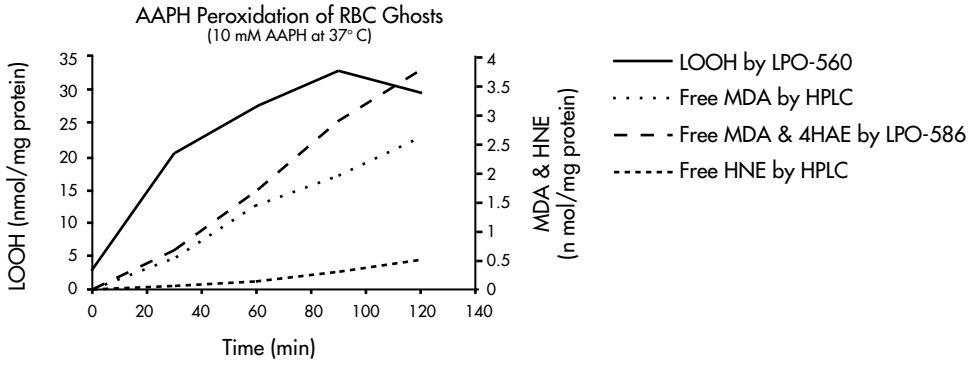
The LPO-586 assay is based on the reaction of a chromogenic reagent, N-methyl-2-phenylindole (R1), with MDA and 4-hydroxyalkenals at 45°C. One molecule of either MDA or 4-hydroxyalkenal reacts with 2 molecules of reagent R1 to yield a stable chromophore with maximal absorbance at 586 nm.



MDA : R = OH
4-hydroxyalkenal : R= hydroxyalkyl

Max = 586 nm

The graph below shows the relationship of LPO-586 lipid peroxidation measurement with other common methods including MDA and HNE by HPLC and lipid hydroperoxide (LOOH) using the OXIS LPO-560 method.



U.S. Patent Number 5, 726, 063.

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LPO-586 Product Summary

Intended Use:	Quantitative measurement of glutathione in biological samples. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	100 test colorimetric
Kit Contents:	• R1 Chromogenic reagent • R2 Acid reagent • S1 Standard (4-HNE-diethylacetal) • S2 Standard (TMOP, Tetramethoxypropane)
Storage and Stability:	24 months from date of manufacture when stored as specified.
Specimen Requirements:	Tissue homogenates and cell lysates.
Reproducibility:	Experiments in which standard samples (0-20 µM) were assayed using the same protocol over a period of 10 days established the standard error of the measurement (SEM) at less than 5%.
Sensitivity:	0.1 nmol/mL assay concentration 0.5 nmol/mL sample concentration

LPO-586 Selected Research Applications:

The ability of reactive oxygen species [e.g., hydroxyl, ($OH^{\bullet}$), peroxyl ($RO^{\bullet}$)] and advanced lipid peroxidation end products (ALE) to react with amino acids/proteins, enzymes and DNA molecules can cause a broad range of pathophysiological conditions in plants, animals and humans. These conditions include fruit ripening disorders (plants), environmental toxicity, fertility, diabetes, cardiovascular disease (e.g. atherosclerosis), neurodegenerative diseases (e.g. Parkinson's and Alzheimer's), arthritis, cancer, inflammation, ischemia/reperfusion, stroke and organ transplantation.

Using the OXIS LPO-586 assay, the Jimenez group researched the oxidative process during tomato fruit ripening. Their data indicated that, under "off-the-vine" ripening conditions (tomatoes were harvested at mature green stage and left at room temperature to ripen), lipid peroxidation in the fruit was increased compared to the vine ripened fruit (1). Ethanol consumption has been reported to cause testicular oxidative injury as measured by lipid peroxidation in a rat model. The condition was positively correlated with decreased fertility and diminished performance of offspring (2). Patients with diabetes mellitus have high incidence of coronary heart disease (CHD) and over 50% of their mortality rate may be due to CHD. In a hyperlipidemic-diabetic hamster model, El-Swefy et al. reported that advanced lipid peroxidation end products (MDA & 4-HAE) were found in LDL and RBC of NIDDM and IDDM hamsters (3). Parkinson's disease is a progressive degenerative disorder of the central nervous system. Using cabegoline (a dopamine agonist), the Finotti group demonstrated in a rat model that lipid peroxidation can be reduced (4). In the brain, cholesterol metabolism can produce LDL and HDL is synthesized by glial cells and associated with amyloid plaques in Alzheimer's disease (AD). Thus, lipid peroxidation is a central feature of vascular illness and may have an important role in AD. Recently, the White group reported that the ability of amyloid precursor protein products from fruit fly, worm, frog, puffer fish, electric ray and humans to induce direct or oxidized lipoprotein mediated neuronal damage *in vivo* will depend on the availability of both Cu and lipoprotein (5). Phenobarbitone (PB) is used for preventing intraventricular hemorrhage, a major complication of pre-birth. The Hodges group used a rat model to show that PB could promote the formation of liver tumors indirectly due to the enhanced production of lipid peroxy radicals. They showed elevated lipid peroxidation levels positively correlated with transient induction of epidermal growth factor inducing S-phase DNA synthesis and subsequent cell proliferation (6). Reperfusion of ischemic tissues causes the generation of reactive oxygen species that contribute to tissue injury. The Ozaki group hypothesized that Rac1, (from the rho family of small GTP-ases) expression increases due to ishemia/reperfusion (I/R) induced necrosis and apoptosis during organ transplantation, myocardial infarction and stroke. Rac1 regulates the production of ROS by an NADPH oxidize in phagocytic and non-phagocytic cells. Using the Bioxytech® LPO-586 assay and others, they demonstrated that targeting inhibition of NADPH oxidase provides a new avenue for *in vivo* therapy aimed at protecting organs at risk from I/R (7).

1. Changes in oxidative processes and components of the antioxidant system during tomato fruit ripening.
Jimenez, A. et al (2002). *Planta* 214: 751-58.
2. Peripubertal paternal EtOH exposure.: testicular oxidative injury, fertility and offspring.
Emaluele NV et al., (2001) *Endocrine* 14: 213-19
3. The effect of Vitamin E, probucol and lovastatin on oxidative status and aortic fatty lesions in hyperlipidermic-diabetic hamster. El-Swefy S et al. (2000). *Atheroscelerosis.* 149: 277-86.
4. Reduction of lipid peroxidation in different rat brain areas after cabergoline treatment.
Finotti, N et al. (2000). *Pharmacol. Res.* 42 (4): 287-87
5. Contrasting species-dependent modulation of copper-mediated neurotoxicity by the Alzheimer's disease amyloid precursor protein. White, AR et al. (2002). *J. Neuroscience* 22 (2): 365-76.
6. Potentiation of Epidermal growth factor or EGF-induced DNA synthesis in rat hepatocytes by phenobarbitone (PB): possible involvement of oxidative stress and kinase activation.

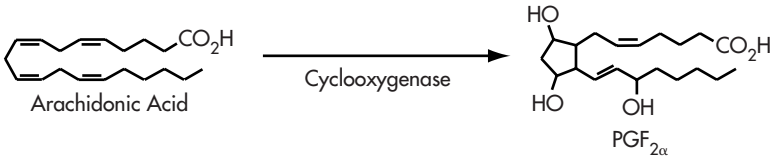
Bioxytech® 8–Isoprostane Assay 21019

Immunoassay For 8–epi–Prostaglandin $F_{2\alpha}$

For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 21019

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is formed naturally from arachidonic acid via the cyclooxygenase (COX I and II) mediated pathways. It functions as a potent vasoconstrictor *in vivo* under the control of COX I and II. Inhibition of COX activity and subsequent production of $PGF_{2\alpha}$ provides the mechanism for the anti-inflammatory properties of common non-steroidal anti-inflammatory drugs (NSAIDS) such as aspirin, ibuprofen and others.



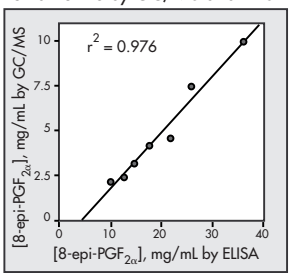
Isoprostanes are prostaglandin-like compounds that are produced upon peroxidation of arachidonic acid. They arise as a result of oxidative insult both *in vivo* and *ex vivo*. Because of this non-enzymatic, oxidative pathway, 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) has become a popular biomarker relating oxidative stress to various disease pathologies. Similar to the COX derived $PGF_{2\alpha}$, 8-epi-$PGF_{2\alpha}$ has been shown to have biological activity as a potent pulmonary and renal vasoconstrictor. The Bioxytech® 8-Isoprostane-EIA provides a simple method for determination of 8-isoprostane (8-epi-$PGF_{2\alpha}$) in biological samples such as tissue, plasma and urine.

8-Isoprostane-EIA Method

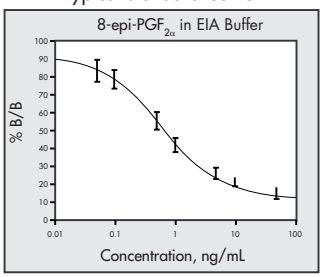
The 8-Isoprostane-EIA assay is a competitive enzyme-linked immuno sorbent assay (ELISA) for determining levels of 8-epi-PG F_{2g} in biological samples. Briefly, samples or standards are added simultaneously with an unbound 8-epi-horseradish peroxidase (HRP) conjugate. Sample and standard 8-epi compete with 8-epi-HRP for binding to the stationary anti-8-epi bound to the plate wells. After washing, a Tetramethyl Benzidine (TMB) substrate is added, resulting in color development. Color intensity is proportional to the amount of 8-epi-HRP bound and inversely proportional to the amount of 8-epi in the samples or standards. The Bioxytech® 8-isoprostane-EIA has a high degree of correlation with the gold standard gas chromatoigraph mass spectrometry (GC/MS) methods.

The Bioxytech® 8-isprostane EIA is recommended for use in tissue, serum or plasma samples and requires solid phase extraction using a C_{12} or silica column prior to sample assay. Although suitable for measurement of 8-isoprostane in urine, OXIS recommends our Urinary 8-isoprostane-EIA method (21048) for this purpose. A third alternative is our new Urinary 8-isoprostane metabolite assay (21049).

Comparison of isoprostane levels in human urine by GC/MS and ELISA



Typical Standard Curve



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21019

8-Isoprostane-EIA Product Summary

Intended Use:	Quantitative measurement of 8-Isoprostane in biological samples. Also known as 8-epi-Prostaglandin $F_{2\alpha}$ and 15-F_{2t}-isoprostane. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	96 Test Colorimetric (ELISA)
Kit Contents:	• 96 Well Anti-8-epi Coated Microtiter Plate • 8-epi Standard • Wash Buffer • Dilution Buffer • TMB Substrate • 8-epi-HRP Conjugate • Reagent Troughs
Storage and Stability:	12 months from date of manufacture when stored as specified.
Specimen Requirements:	Tissue, plasma, and urine. (Recommended for plasma and tissue)
Specificity:	Cross-reactivity at 50% B/B_0

8-epi-$PGF_{2\alpha}$	100%
9a,11b-Prostaglandin $F_{2\alpha}$	4.1%
13,14-Dihydro-15-keto $PGF_{2\alpha}$	3.0%
9b,11a-Prostaglandin $F_{2\alpha}$	<.01%
Prostaglandin $F_{2\alpha}$	<.01%
Prostaglandin E_2	<.01%
Prostaglandin D_2	<.01%
Arachidonic Acid	<.01%

Sensitivity:	0.05 ng/mL (precision best on steeper, linear portion of the curve)

8-Isoprostane-EIA Selected Research Applications

The OXIS 8-isoprostane assay has been used to study a variety of diseases and parameters including circadian rhythms as related to uric acid and lipid peroxidation in diabetic and "healthy" men and the effects of cigarette smoking on oxidative stress parameters related to Chronic obstructive Pulmonary Disease (COPD) related to emphysema. In other cardiovascular related studies, researchers have used the OXIS kit to look at the effects of certain lipid lowering therapies (e.g. HMH-CoA inhibitor/Cerivastatin) on plasma lipid peroxidation.

Kanabrocki, E.L., et. al., showed that oxygen-based free radicals, such as $^{\cdot}OH$, $O_2^{-\cdot}$, may be involved in the peroxidation of lipids, which was enhanced in the diabetic subjects, but that $ONOO^-$ is unlikely to make a major contribution. Although higher levels of uric acid in the diabetics helped to scavenge out $ONOO^-$, it was ineffective in preventing lipid peroxidation (1).

SLPI, like alpha1-anti-proteinase loses its activity due to oxidative stress. This results in enhanced proteolysis of lung connective tissue (e.g., elastin), a key event in the pathogenesis of emphysema. Cavarra E. et al. showed that 8-isoprostane levels were correlated with decreased antitrypsin activity among smokers illustrating some of the manifestation of smoking induced oxidative stress (2). Lipid-lowering therapy, using HMH-CoA inhibitor/Cerivastatin decreases the risk of coronary events in both primary and secondary prevention. Tsunekawa T et al. showed that plasma 8-isoprostane was decreased with the treatment (3).

1. Circadian variation in oxidative stress markers in healthy and type II diabetic men.
Kanabrocki, E.L., et. al., (2002) Chronobiology International 19(2):423-39

2. Human SLPI (secretory leukoprotease inhibitor) inactivation after cigarette smoke exposure in new in vivo model of pulmonary oxidative stress.
Cavarra E.. et al. (2001). Am. J. Physiol Lung Cell Mol. Physiol 282: L412-L417

3. Cerivastatin, a hydroxymethylglutaryl coenzyme A reductase (HMG-CoA) inhibitor, improved endothelial function in elderly diabetic patients within 3 days.
Tsunekawa T et al. (2001). Circulation. 104(4):376-379

Bioxytech® H_2O_2–560 Assay 21024

Quantitative Measurement of Hydrogen Peroxide in Aqueous Solutions

For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 21024

Hydrogen peroxide is a powerful oxidant at the center of many redox pathways.

It is also a key player in the healing process as neutrophils gather at a wound site post trauma and release bactericidal reactive oxygen species (ROS) and H_2O_2 to kill bacteria and prevent infection. The ongoing interest in understanding the role of hydrogen peroxide in biological systems including its role as a second messenger prompts the need for a rapid and quantitative measurement tool. Researchers also require a method of hydrogen peroxide measurement for laboratory reagents and detergents in order to prevent the unintended contribution of peroxidation by reagent use. The Bioxytech® H_2O_2-560 assay allows for simple colorimetric measurement of hydrogen peroxide.

H_2O_2-560 Method

The Bioxytech® H_2O_2-560 Assay is based on the oxidation of ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}) by hydrogen peroxide under acidic conditions, equation (1). The ferric ion binds with the indicator dye xylenol orange (3,3′-bis[N,N-di(carboxymethyl)-aminomethyl]-o-cresolsulfone-phthalein, sodium salt) to form a stable colored complex which can be measured at 560 nm, equation (2).

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^{\bullet} + OH^{-} \quad (1)$$

$$Fe^{3+} + XO \longrightarrow Fe^{3+}\text{-}XO \quad (2)$$

The Fe^{3+}-xylenol orange complex has an extinction coefficient of $1.5 \times 10^4\ M^{-1}\cdot cm^{-1}$ at 560nm when ferric ions are added in the absence of hydrogen peroxide. However, in the presence of sorbitol there is substantial chain oxidation of ferrous ion, increasing the color yield. This results in an apparent extinction coefficient of $2.67 \times 10^5\ M^{-1}\cdot cm^{-1}$, indicating that approximately 18 moles of Fe^{2+} are oxidized to Fe^{3+} for every mole of hydrogen peroxide present, increasing the sensitivity of the assay.

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21024

H_2O_2-560 Product Summary

Intended Use:	Quantitative measurement of hydrogen peroxide. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	100 Test Colorimetric
Kit Contents:	• R1, Ammonium Iron Sulfate (Fe^{2+}) • R2, Sorbitol/Xylenol Orange solution
Storage and Stability:	12 months from date of manufacture when stored as specified. Working reagent stable 12 hours refrigerated.
Specimen Requirements:	Aqueous samples
Sensitivity:	1 uM in the assay

H_2O_2-560 Selected Research Applications

The OXIS H_20_2-560 assay has been used to assess endogenous production of the pro-oxidant hydrogen peroxide as well as to monitor levels of exogenously applied H_20_2 in various research applications.

Joo et al. studied the relationship of gravitropism in plant roots to ROS production. They showed increased H_20_2 production in various regions of the root and suugested this could play a role in early gravitropic induced root response (1).

Kaul et al. found that the endogenous antioxidant activities (e.g. SOD, catalase and GSH) are not inhibited by rhinovirus infection, suggesting that oxidative stress is a result of increased production of pro-oxidants. The OXIS H_2O_2-560 kit was used to measure the H_2O_2 production in cell culture supernatant as a means of monitoring pro-oxidant production (2).

LeVine et al. evaluated hydrogen peroxide production by macrophages in rat bronchoalveolar lavage (BAL) using the OXIS H_2O_2-560 assay (3). They utilized murine surfactant knockout models to show that both surfactant protein A and D play an apparent role in reducing inflammation as measured by decreased H_20_2 in SP-A and SP-D deficient mice.

Avshalumov et al. tested Long-Evans Rat Hippocampal slices for evoked potential in the presence of exogenously provided H_2O_2. Hydrogen peroxide was evaluated in the applied media using the OXIS H_2O_2-560 assay (4).

1. Joo JH et al. Role of auxin-induced reactive oxygen species in root gravitropism.
Plant Physiology, (2002) 126:1055-1060.

2. Rhinovirus-induced oxidative stress and interleukin-8 elaboration involves p47-phox but is independent of attachment to intercellular adhesion molecule-1 and viral replication.
Kaul, P et al. (2000). J. Infec. Dis. 181:1885-90.

3. Distinct effects of surfactant protein A or D deficiency during bacterial infection on the lung.
Le Vine, AM et al. (2000). J. Immunol. 165:3934-40.

4. Mechanism underlying H_2O_2-mediated inhibition of synaptic transmission in rat hippocampal slices. Avshalumov MV et al., (2000). Brain Research (2000) 882: 86-94.

Bioxytech® LPO–560 Assay 21025

Quantitative Measurement of Lipid Hydroperoxides (LOOH)

For Research Use Only, Not Intended For Use in Diagnostic Procedures

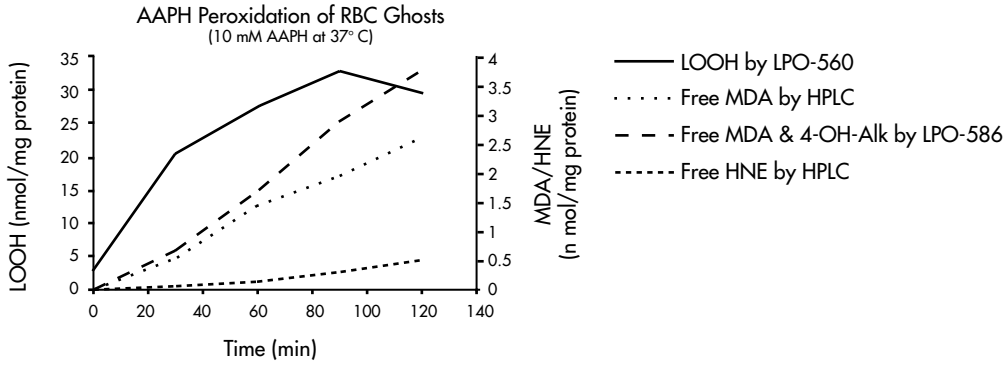
Catalog Number 21025

Lipid peroxidation (LPO), which is associated with oxidative stress, contributes to pathological processes in aging and many diseases such as atherosclerosis, diabetes, arthritis and Alzheimer's disease. Oxidation of polyunsaturated lipids involves an allylic hydrogen abstraction, insertion of molecular oxygen, and subsequent abstraction of hydrogen by the resulting hydroperoxyl radicals to form lipid hydroperoxides (LOOHs). The measurement of LOOHs are extensively used as an indicator of the LPO reaction. Some techniques, such as HPLC-chemiluminescence, thiobarbituric acid conjugation of MDA, iodide oxidation, and conjugated diene formation etc., have been used for the determination of LOOH. However, these methods either require sophisticated instrumentation or lack specificity and sensitivity. Therefore, the LPO-560 assay is designed to detect total LOOHs in plasma by a simple and sensitive method. An advantage of the LPO-560 assay is that total available plasma or serum LOOHs can be determined without involvement of a lipid extraction step.

LPO-560 Method

The design concept is based on the theory that hydroperoxides can be determined by the Ferrous Oxidation-Xylenol Orange (FOX) assay in conjunction with the reductant tris(2-carboxyethyl) phosphine (TCEP). Catalase is first used to decompose the existing hydrogen peroxide in biological samples.

The graph below shows the relationship of LPO-560 lipid peroxidation measurement with other common methods including MDA and 4HNE by HPLC and MDA and HAE using the OXIS LPO-586 method.



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21025

LPO-560 Product Summary

Intended Use:	Quantitative measurement of total LOOH. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	100 test colorimetric
Kit Contents:	• Enzyme • Reducing Agent • Chromagen • Color Developer • Buffer
Storage and Stability:	24 months from date of manufacture when stored in the dark at 2° – 8° C.
Specimen Requirements:	Heparinized plasma, serum or tissue homogenates

Assay Precision:

n=26	*tert*-Butyl Hydroperoxide Low	Med	High
Average (µM)	1.94	3.80	10.0
Intra-assay (% CV)	3.64	2.82	2.41
Inter-assay (% CV)	4.67	6.08	3.10
Total Precision (% CV)	5.31	6.39	3.53

Sensitivity: 1.2 µM in original sample

LPO-560 Selected Research Applications

This popularity of this assay has increased among researchers seeking an alternative marker other than the more commonly looked at malondialdehyde (MDA). While originally marketed as an acute LPO plasma assay, researchers have found the kit useful for samples as diverse as cell culture supernatant to coral polyps.

In a cardiovascular related study investigating the effect of remnant lipoproteins (RLPs) on plasma redox status Doi et al.. evaluated levels of lipid hydroperoxides (LOOH) in cell culture supernatants. They showed that LOOH levels were dramatically increased in the cell culture supernatant of HUVECs incubated with RLPs compared to controls.

Williams et al. evaluated the safety and antioxidant potential of Lipoic acid (LA), a water-soluble and fat-soluble antioxidant, in horses undergoing light voluntary pasture exercise. They tested for endogenous antioxidant status (i.e. cGPX and GSH) using the OXIS cGPx and GSH-420 kits with the automated OxyScan™ Analyzer and lipid hydroperoxides using the OXIS LPO-560 Assay (3). They showed that LA supplemenation of 10 mg/kg did not result in any adverse signs/effects over a 14-day period and moderately reduced the oxidative stress of horses that were allowed light activity.

Downs, et al. studied the oxidative stress in corals and showed that coral (Zooxanthelee) bleaching may be a final strategy to defend coral from oxidative stress. High levels of antioxdant enzymes and heat shock proteins were negatively correlated with levels of oxidative damage products including LOOH measured using the OXIS LPO-560 assay.

Wang et al. investigated the shortened lifespan of insect cell lines due to viral infection, a necessary part of recombinant protein expression. Using the LPO-586 assay they showed that oxidative stress is increased upon infection leading a postulation that lipid peroxidation can cause "leaky membranes" leading to cell death (4).

1. Remnant lipoprotein induce proatherothrombogenic molecules in endothelial cells through a redox-sensitive mechanism. Doi, H et al. (2000) Circulation 102: 670-6

2. Lipoic acid as an antioxidant in mature thoroughbred geldings: A preliminary study. Williams, C.A. et. al., (2002). J. Nutr. 132: 1628S-1631S

3. Oxidative stress and seasonal coral bleaching. Downs, CA et al,. (2002). Free Radic. Biol. Med. 33(4):533-543

4. Evidence of oxidative stress following the viral infection of two lepidopteran insect cell lines Wang et al. (2001) Free Radical Biology and Medicine 31:1448-1455.

Bioxytech® 8-OHdG Assay 21026

Immunoassay for 8-Hydroxy-2'-deoxyguanosine (8-OHdG)

For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 21026

In recent years a number of studies have correlated elevated levels of oxidative mediated DNA damage to various disease conditions. This has created a demand for easy to measure biomarkers that can be used to monitor oxidation of DNA in biological samples. 8-Hydroxy-2'-deoxyguanosine (8-OHdG) is a modified base that occurs in DNA after attack by reactive oxygen species (ROS), most notably hydroxyl (OH) radical. 8-OHdG modified bases are normally repaired by excision of the modified base resulting in formation of 8-hydroxy-2′-deoxyguanosine (8-OHdG).

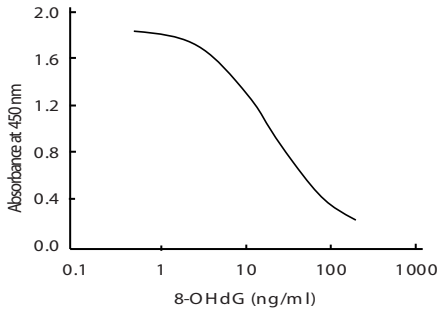
8-OHdG can be identified through Southern blotting and tissue IHC. It can be found in plasma and in higher concentrations in the urine using HPLC or other means. For this reason it has become a very popular biomarker used to correlate oxidative stress with damage to DNA. The OXIS Kit allows for quantitative measurement of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in tissue, serum, plasma, urine and cell culture supernatant.

8-OHdG-EIA Method

The Bioxytech® 8-OHdG-EIA kit is a competitive enzyme-linked immunosorbent assay (ELISA). Briefly, 8-OHdG antibody (anti-8-OHdG) is added to wells along with sample or standard. "Free" 8-OHdG in the sample or standards competes with "stationary" (plate bound) 8-OHdG for the anti-8-OHdG binding sites. Therefore, *increased* sample 8-OHdG concentrations leads to *decreased* plate bound anti-8-OHdG.

After "free" anti-8-OHdG/8-OHdG complexes are washed from the plate, the stationary 8-OHdG/anti-8-OHdG complexes are then treated with an enzyme labeled secondary antibody. Subsequent addition of a chromagenic substrate allows quantification of plate bound anti-8-OHdG. The resulting color is inversely proportional to the original sample 8-OHdG.

Typical Standard Curve



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21026

8-OHdG-EIA Product Summary

Intended Use:	Quantitative measurement of 8-hydroxy-2′-deoxyguanosine. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	96 Test colorimetric (ELISA)
Kit Contents:	• 96 well 8-OHdG coated microtiter wells • Primary 8-OHdG antibody • Primary antibody dilution buffer • Secondary antibody • Secondary antibody dilution buffer • Chromagen • Chromagen dilution buffer • Wash buffer • Standard
Storage and Stability:	12 months from date of manufacture when stored as specified.
Specimen Requirements:	Serum, plasma, urine and DNA that has been extracted and digeted.
Specificity:	Specific for DNA adduct 8-hydroxydeoxyguanosine. No cross-reactivity with other known 8-OHdG analogues was observed.
Sensitivity:	Threshold detection limit of 0.6 ng/mL

8-OHdG-EIA Selected Research Applications

The OXIS 8-OHdG ELISA assay kit has been used for a number of research applications in both human and veterinary related topics including studying the effect of antioxidant supplementation in high performance animals (e.g. sled dogs). In human studies the kit has been used to look at circadian variations in markers of oxidative stress in diabetic men as well as the effect of prolonged aerobic exercise on human immune function.

Baskin et al. showed that dietary antioxidant supplementation for exercising sled dogs can decrease levels of oxidative stress and subsequent levels of DNA damage as measured by decreased 8-OHdG in supplemented animals vs increased 8-OHdG in control animals (1). Kanabrocki et al used urinary 8-OHdG as an index of peroxynitrite mediated damage to DNA. They showed that oxygen-based free radicals, such as $^{\cdot}OH$, $O_2^{-\cdot}$, may be involved in the peroxidation of lipids which was enhanced in the diabetic subjects, but that $ONOO^-$ (causes oxidative damage in DNA or 8-OHdG pools) is unlikely to make a major contribution. Although higher levels of uric acid in the diabetics helped to scavenge out $ONOO^-$ as measured by decreased 8-OHdG, it was ineffective in preventing lipid peroxidation. Overall there appeared to be a statistically significant circadian variation of fluctuating baseline 8-OHdG levels and uric acid levels (i.e elevated uric acid levels correspond to decreased basal 8-OHdG levels) (2). Tsai et al. showed that 8-OHdG is increased during prolonged human aerobic exercise (e.g. as measured in marathon runners) and remains elevated for up to a week post exercise. The authors note that this may be responsible in part for exhaustive related immune dysfunction (3).

1. Effects of dietary antioxidant supplementation on oxidative damage and resistance to oxidative damage during prolonged exercise in sled dogs.
Baskin CR et al. (2000) Am. J. Vet Res. 61(8): 886-91

2. Circadian variation in oxidative stress markers in healthy and type II diabetic men.
Kanabrocki, E.L., et. al., (2002) Chronobiology International 19(2):423-39

3. Oxidative DNA damage in human peripheral leukocytes induced by massive aerobic exercise.
Tsai, K. et al. (2001). Free Radic. Biol. Med. 31(11):1465-72

Aconitase-340 Assay 21041

Quantitative Measurement of Aconitase Enzyme Activity

For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 21041

There are three biological components most commonly looked at in assessing oxidative insult: lipid peroxidation, protein oxidation and DNA oxidation. While excellent means for assessing lipid peroxidation and DNA oxidation exist, assessment of protein oxidation has been problematic. Carbonylated proteins, once thought to be a very useful marker, have not proven adequate due to sample instability and reproducibility problems.

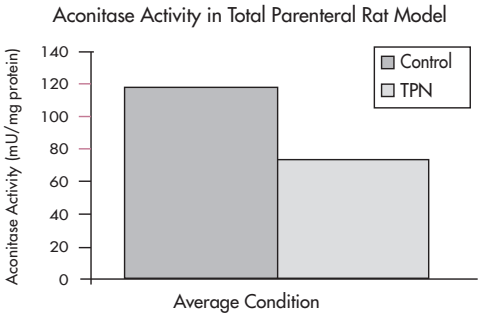
According to recent literature, decreased aconitase enzyme activity is a sensitive and specific indicator of oxidative damage during aging, Parkinson's and other disease progression. The function of aconitase is to isomerize citrate to isocitrate a key intermediate of the citric acid cycle. Because of its role in cellular energy production, aconitase enzyme function is well positioned as an important marker relative to biological decline.

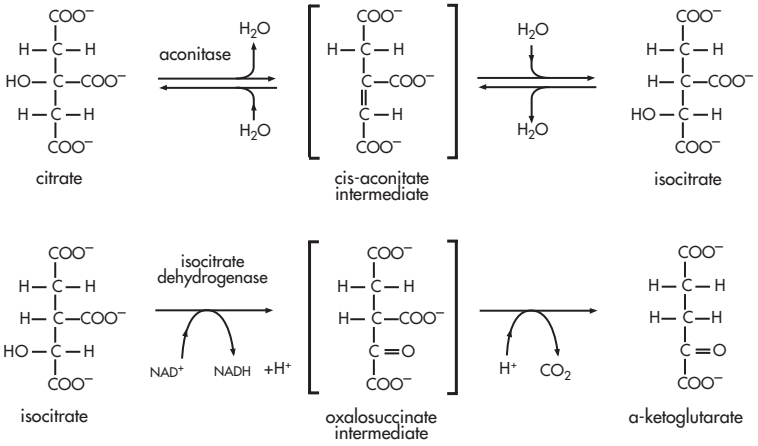
Two forms of aconitase are known (mitochondrial and cytosolic). The cytosolic form is a bifunctional protein. The holo (4Fe/4S) form has aconitase activity while the fully apo (iron-free) form, known as Iron Regulatory Protein-1 (IRP-1) is devoid of enzymatic activity but plays an important role in regulating the intracellular iron level. In a complete cellular homogenate, both mitochondrial and cytosolic forms will contribute to the total aconitase activity, with the mitochondrial aconitase being predominant in most tissue types.

Aconitase-340 Method

The *Oxis*Research® Aconitase-340 assay utilizes the coupled reaction of citrate to isocitrate and isocitrate to α-ketoglutarate as the basis for quantitating aconitase enzyme activity.

Under the assay conditions, the rate of NADH production is a measure of aconitase activity. To test the performance of the OXIS kit on biological samples, aconitase activity was measured in liver samples from a parenterally fed rat model. Using the Aconitase-340 assay, activity levels of parenterally fed animals were significantly ($P = 0.0002$) reduced compared to controls.





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2. Gardner, PR and Fridovich, I, (1992) Inactivation-Reactivation of Aconitase In E Coli: A Sensitive Measure of Superoxide Radical, J. Biol. Chem. 267: 8757-8763.
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21041

Aconitase-340 Product Summary

Intended Use:	Quantitative measurement of aconitase enzyme activity. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	100 test colorimetric.
Kit Contents:	• Assay Buffer • Substrate • Enzyme • NAD^+ • Standards
Storage and Stability:	12 months from date of manufacture when stored as specified.
Specimen Requirements:	Tissue or cultured cells.

Assay Precision:	Aconitase in buffer		Biological
n=18	Low	High	Rat Lung
Mean (A 340 /min)	0.0052	0.0254	0.0049
Intra-assay (% CV)	5.11	1.63	3.64
Inter-assay (% CV)	4.82	3.53	8.63
Total Precision (% CV)	6.00	3.71	9.01

Sensitivity: 0.625 mU/ mL in the reaction mixture.
2.5mU/ mL in original sample.

Aconitase-340 Selected Research Applications

The OXIS assay for aconitase enzyme activity is gaining favor among researchers looking for novel ways to quantify the oxidation of proteins. The assay has not been on the market long enough to show many citations but here we highlight one that has come to our attention.

Doxorubicin is an anthracyclin antibiotic sometimes often used in treatment of human neoplasmas such as lymphomas, acute leukemias, stomach, breast, ovarian and cancers of the bone. Its clinical use is limited in that it may also induce cardiotoxicity. Childs, Leeuwenburgh et al. investigated the mechanisms of this cardiotoxicity employing the OXIS Aconitase-340 assay in the process. Although not statistically significant, they showed aconitase activity was decreased in the heart of Doxorubicin treated rats compared to controls (1). They also showed elevated serum levels of free iron. It is interesting to note that aconitase is also an iron storage protein but it is unknown whether its deactivation contributed to the elevated free iron in the serum.

1. Doxorubicin treatment *in-vivo* causes cytochrome c release and cardiomyocyte apoptosis, as well as increased mitochondrial efficiency, superoxide dismutase activity and Bcl-2:Bax ratio.
Childs, et al. (2002) Cancer Research 62:4592-4598.

Bioxytech® HAE-586 Assay 21043

Quantitative Measurement of 4-Hydroxyalkenals

For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 21043

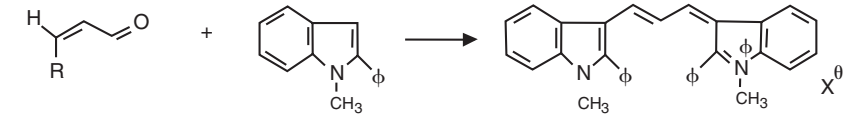
Lipid peroxidation, a well established mechanism of cellular injury in both plants and animals, is used as an indicator of oxidative stress in various biological samples. Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds including malondialdehyde (MDA) and 4-hydroxyalkenals (4-HAE). The most abundant 4-hydroxyalkenal formed by lipid peroxidation is 4-hydroxynonenal (4-HNE). HNE adducts, which are cytotoxic and mutagenic, can alter signal transduction and gene expression. 4-HNE as a biomarker has implications in the areas of neurodegenerative disease, atherosclerosis and nutrition research relative to food freshness and product stability.

The Bioxytech® LPO-586 from *Oxis*Research® has traditionally been used to measure either the sum of MDA and 4-hydroxyalkenal or MDA only. In response to an increased interest in 4-HNE, *Oxis*Research® has developed a new assay specific for the HAE component of lipid peroxidation by-products.

HAE-586 Method

The *Oxis*Research® HAE-586 assay employs an extraction step that allows quantitative separation and detection of HAE in the presence of excess MDA. After sample extraction, reaction of N-methyl-2-phenylindole (NMPI) with hydroxyalkenals yields a stable carbocyanine dye visible at 586 nm.

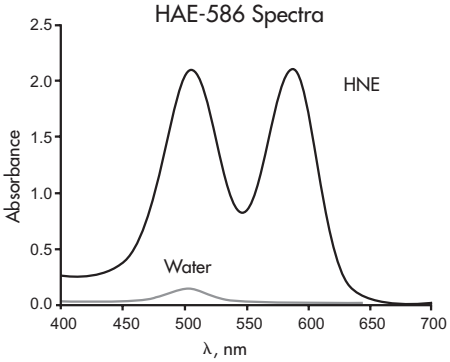
The by-products of the reaction also react to form a stable chromophore visible at 505nm. The graph below shows the characteristic spectra obtained from the HAE-586 assay.



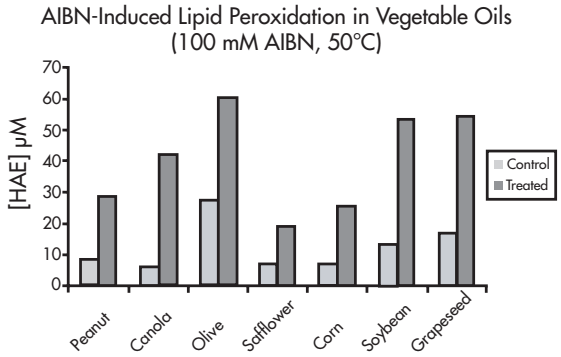
4-Hydroxyalkenal : R = Hydroxyalkyl

NMPI

Carbocyanine Dye



As an example of the assay's usefulness, a study was designed to test the HAE content of various cooking oils and their susceptibility to lipid peroxidation. The data shows the ability of the HAE-586 assay to detect sample HAE content.



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21043

HAE-586 Product Summary

Intended Use:	Quantitative measurement of 4-hydroxyalkenals. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	100 test colorimetric.
Kit Contents:	• N-Methyl-2-phenylindole • Methanesulfonic acid • HNE Standard • Butylated Hydroxytoluene (BHT) • Methanol • Dichloromethane
Storage and Stability:	12 months from date of manufacture when stored as specified.
Specimen Requirements:	Cell Lysate & Tissue

Assay Precision:

n=10	Low	Med.	High
Mean (µM)	0.99	1.79	4.30
Intra-assay (%CV)	5.1	2.7	2.9
Total Precision (%CV)	5.9	4.2	3.3

Sensitivity:

LLD in reaction mixture	0.0743 µM
LLD A586 in sample	0.0078

HAE-586 Selected Research Applications

Parikh et al studied endogenous antioxidant activities and lipid peroxidation (noting that EPUFAs in the brain are unstable and decompose, preferentially forming HAE's) in the brain of rats treated with various antipsychotic drugs. They determined that chronic Haloperidol treatment had the effect of reducing catalase, SOD and GPx activities (1). The OXIS HAE-586 kit was used to show that hydroxyalkenal (HAE) formation was increased in parallel with the decreased endogenous antioxidant activities in Haloperidol treated animals. These trends were not observed under treatment with atypical antipsychotic drugs such as Clozapine, Risperidone and Olanzapine.

1. Differential effects of antipsychotics on expression of antioxidant enzymes and membrane lipid peroxidation in rat brain. Parikh, V. et al. (2003) J. Psychiatric Research 37:43-51

Bioxytech® MDA-586 Assay 21044

Quantitative Measurement of Malondialdehyde (MDA)

For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 21044

Lipid peroxidation, a well-established mechanism of cellular injury in both plants and animals, is used as an indicator of oxidative stress. Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds including reactive carbonyl compounds. The most abundant of these is malondialdehyde (MDA), which is commonly used as a biomarker of lipid peroxidation. The MDA-586 from *Oxis*Research® is designed to measure free or total MDA (i.e. free and protein bound Schiff base conjugates) under assay conditions that minimize interference from other lipid peroxidation products such as 4-hydroxyalkenals. The assay improves upon our popular Bioxytech® LPO-586 assay system.

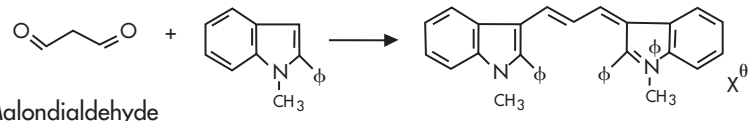
Improvements over existing method:

- Acid hydrolysis defined for "total MDA" measurement.
- Enhanced sensitivity via 3rd derivative spectroscopy.
- Antioxidant reagent included.
- Acid reagent included.

The MDA-586 allows for simple, rapid measurement of MDA with superior specificity and reproducibility compared to other commonly used methods such as the thiobarbituric acid reactive substance assay (TBARS).

MDA-586 Method:

The *Oxis*Research® MDA-586 method is based on the reaction of a chromogenic reagent, N-methyl-2-phenylindole (NMPI), with MDA at 45°C to form an intensely colored carbocyanine dye with a maximum absorption at 586 nm.



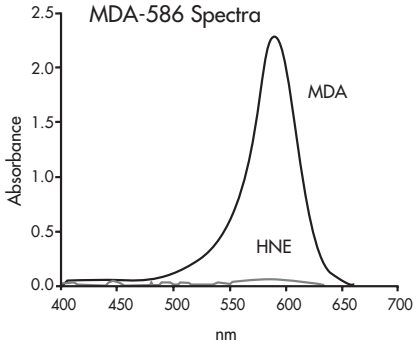
Malondialdehyde (MDA) NMPI Carbocyanine Dye

The reaction is carried out in hydrochloric acid with the addition of an antioxidant to further minimize the reaction of 4-hydroxyalkenals. Under these conditions, there is little absorbance at 586 nm from HNE, the most common 4-hydroxyalkenal produced in cells subjected to lipid peroxidation.

The table below shows improved standard deviation (SD) and lower limit of detection (LLD) values for MDA assayed in plasma utilizing the MDA-586 3rd derivative spectroscopy prodedure.

	Zero Derivative	Third Derivative
SD	0.65 µm	0.06 µm
LLD	3.0 µm	0.2 µm *

*LLD is 0.1 µm when four replicates used.



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21044

MDA-586 Product Summary

Intended Use:	Quantitative measurement of malondialdehyde. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	100 test colorimetric
Kit Contents:	• N-methyl-2-phenylindole • Concentrated hydrochloric acid • MDA Standard (tetramethoxypropane, TMOP) • Butylated hydroxytoluene (BHT) • Probucol • Methanol
Storage and Stability:	12 months from date of manufacture when stored as specified.
Specimen Requirements:	Serum, plasma, cell lysate & tissue

Assay Precision:

n=10	Low	Med.	High
Mean (µM)	1.04	1.99	4.79
Intra-assay (%CV)	3.4	1.2	1.6
Total Precision (%CV)	3.7	1.6	1.7

Sensitivity:

LLD in reaction mixture	0.0801µM
LLD A_{586} in sample	0.0088

MDA-586 Selected Research Applications

The OXIS MDA-586 assay is still rather new but fast gaining popularity among researchers needing an easy, highly specific method of malondialdehyde (MDA) determination. Here we present a very nice piece of literature by two prominent groups in which the assay was used to evaluate plasma MDA levels.

Dr. Jason D. Morrow and Dr. Lester Packer groups lead by Dr. Block carried out a nationwide study on oxidative stress in human populations. Along with other biomarkers, they looked at plasma MDA levels among different population sets: smokers/non-smokers, age, sex, ethnicity, alcoholic/non-alcoholic etc.

The authors showed that differences in plasma MDA levels were statistically significant in comparisons of smokers vs. nonsmokers, race and the sexes. No significant differences were reported comparing age, weight and alcohol consumption. The authors stated that the OXIS MDA-586 method for measuring MDA in plasma was equally competitive to HPLC method (1).

1. Factors associated with oxidative stress in human populations. Block G et al. (2002). Am. J. Epidemology 156(3):274-285

α_1AP-410 Assay

21047

Quantitative Measurement of α_1Antiproteinase Enzyme Activity

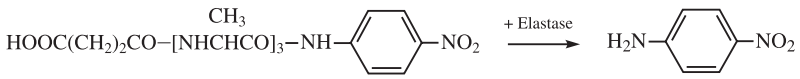
For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 21047

The most common indicators of oxidative damage are lipid peroxidation, protein oxidation and DNA oxidation. Until recently, the main test for protein oxidation has been carbonylated protein assessment. While sometimes useful, tedious procedures and reproducibility issues have lead researchers to seek alternative methods. For this reason *Oxis*Research® has sought to provide sensitive assays allowing assessment of specific protein oxidation through measurement of enzyme activities shown to be affected by reactive oxygen species (ROS) and Reactive Nitrogen Species (RNS). The first kit focused on the enzyme aconitase. That assay has the most utility in mitochondria rich tissues. The need and demand for a systemic marker indicative of protein oxidation has prompted the development of the $\alpha_1$anti-proteinase (α_1AP) activity assay. Excess ROS and RNS can inactivate α_1AP through oxidation of essential methionines. α_1AP has been shown to be very sensitive to peroxynitrite, hydroxyl radicals and hypochlorous acid. It is well positioned as a key biomarker in the assessment of oxidative stress due to inflammation, disease and other at risk factors in biological systems.

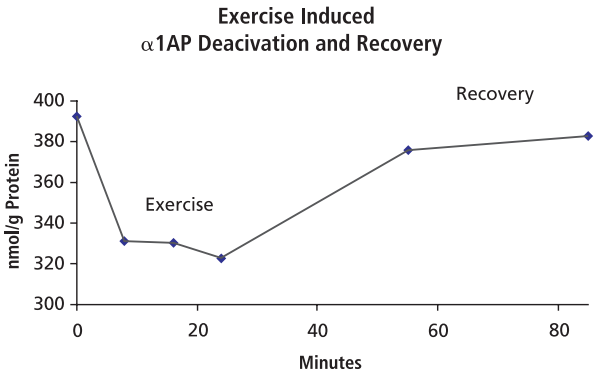
α_1AP-410 Method

The Bioxytech® α_1AP-410 Assay is based on the inactivation of elastase by α_1AP. Since α_1AP is an irreversible, equimolar inhibitor of elastase, the concentration of active α_1AP is defined as the molar decrease in elastase activity. The difference between control (elastase alone) and test (elastase plus sample α_1AP), in the presence of a substrate is used to calculate α_1AP activity in the samples. Elastase inactivation is measured by monitoring the rate of cleavage of the substrate N-succinyl-(Ala)3-ρ-nitroanalide (NSAN). Cleavage of NSAN results in production of a chromagen, ρ-nitroaniline, having a maximal absorbance wavelength at 410nm.



N-Succinyl-(Ala)3-*p*-nitroanilide *p*-Nitroaniline

Serum, plasma and other bodily fluids including sputum, synovial fluid and fluid from sites of inflammation are sources of a_1AP. Some tissues such as lung, liver and pancreas also contain a_1AP making the assay useful in these tissues as well. The concentration of a_1AP activity in normal human plasma is 20- 50 µM. The graph to the right shows the assay's utility in monitoring free radical activity in a time-course exercise related study using plasma samples.



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2. Fujita J *et al*. (1990) Evaluation of elastase and antielastase balance in patients with chronic bronchitis and pulmonary emphysema *Am Rev Respir Dis* 1 42, 57-62.
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4. Whiteman M *et al*. (1999) Modulation of peroxynitrite- and hypochlorous acid-induced inactivation of α_1-antiproteinase by mercaptoethylguanidinel *British J of Pharmacology* 1 26, 1646-1652.
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21047

α_1AP-410 Product Summary

Intended Use:	Quantitative measurement of α_1AP enzyme activity. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	100 test colorimetric
Kit Contents:	• Assay Buffer • Substrate • Enzyme
Storage and Stability:	9 months from date of manufacture when stored as specified.
Specimen Requirements:	Plasma, serum, tissue or cultured cells.

Assay Precision:

n=23	Low	Medium	High
Mean (µM)	0.50	0.79	1.29
Intra-assay (% CV)	5.88	5.30	1.08
Inter-assay (% CV)	4.46	4.00	0.23
Total precision (% CV	6.67	6.01	1.09

Sensitivity:

LLD in Reaction Mixture	0.025 µM
LLD in Sample	0.5 µM

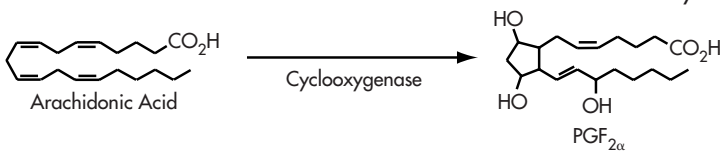
Bioxytech® Urinary 8-EPI-PGF$_{2\alpha}$ Assay 21048

Immunoassay for 8-epi-prostaglandin F$_{2\alpha}$ in Urine Samples

For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 21048

Prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) is formed naturally from arachidonic acid via the cyclooxygenase (COX I and II) mediated pathways. It functions as a potent vasoconstrictor *in vivo* under the control of COX I and II. Inhibition of COX activity and subsequent production of PGF$_{2\alpha}$ provides the mechanism for the anti-inflammatory properties of common non-steroidal anti-inflammatory drugs (NSAIDS) such as aspirin, ibuprofen and others.

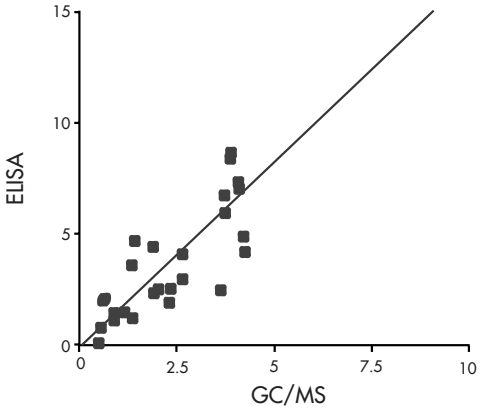


Isoprostanes are prostaglandin-like compounds that are produced upon peroxidation of arachidonic acid. They arise as a result of oxidative insult both *in vivo* and *ex vivo*. Because of this non-enzymatic, oxidative pathway, 8-isoprostane (8-epi-PGF$_{2\alpha}$) has become a popular biomarker relating oxidative stress to various disease pathologies. Similar to the COX derived PGF$_{2\alpha}$, 8-epi-PGF$_{2\alpha}$) has been shown to have biological activity as a potent pulmonary and renal vasoconstrictor. In particular urinary isoprostane is well positioned as a non-invasive test for lipid peroxidation associated with oxidative stress. The Bioxytech® Urinary 8-Isoprostane-EIA provides a simple method for determination of 8-isoprostane (8-epi-PGF$_{2\alpha}$) in urine without the need for sample extraction.

Urinary 8-Isoprostane-EIA Method

The Urinary 8-Isoprostane-EIA assay is a competitive enzyme-linked immuno-sorbent assay (ELISA) for determining levels of 8-epi-Prostaglandin F$_{2\alpha}$ (8-epi) in biological samples. The kit utilizes a pretreatment reagent that does away with the need for sample extraction prior to assay. Briefly, pre-treated samples or standards are added simultaneously with a "free" 8-epi-horseradish peroxidase (HRP) conjugate. Sample or standard 8-epi competes with 8-epi-HRP for binding to the stationary anti-8-epi bound to the plate wells. After washing, Tetramethyl Benzidine (TMB) substrate is added resulting in color development. Color intensity is proportional to the amount of 8-epi-HRP bound and inversely proportional to the amount of 8-epi in the samples or standards. The Bioxytech® 8-isoprostane-EIA has a high degree of correlation with the gold standard Gas Chromatograph/Mass Spectrometry (GC/MS) methods.

Correlation of ELISA and GC/MS Methods



The Bioxytech® Urinary 8-isprostane EIA is strongly recommended for use in urine and does not require solid phase extraction prior to sample assay. For measurement of 8-isprostane parent compound please refer to our products 21019, 8-isoprostane EIA.

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21048

Urinary 8-Isoprostane-EIA Product Summary

Intended Use:	Quantitative measurement of 8-Isoprostane in urine samples. Analyte also known as 8-epi-Prostaglandin $F_{2\alpha}$ and 15-F_{2t}-isoprostane. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	96 Test Colorimetric (ELISA)
Kit Contents:	• 96 Eell Snti-8-epi Coated Microtiter Plate • 8-epi Standard • Pretreatment Reagent • Wash Buffer • Dilution Buffer • TMB Substrate • 8-epi-HRP Conjugate • Reagent Troughs • Stop Solution
Storage and Stability:	12 months from date of manufacture when stored as specified.
Specimen Requirements:	Urine
Specificity:	Cross-reactivity at 50% B/B_0

8-epi-$PGF_{2\alpha}$	100%
9a,11b-Prostaglandin $F_{2\alpha}$	4.1%
13,14-Dihydro-15-keto $PGF_{2\alpha}$	3.0%
9b,11a-Prostaglandin $F_{2\alpha}$	<.01%
Prostaglandin $F_{2\alpha}$	<.01%
Prostaglandin E_2	<.01%
Prostaglandin D_2	<.01%
Arachidonic Acid	<.01%

Sensitivity:	0.05ng/mL (precision best on steeper, linear portion of the curve)

Bioxytech® Urinary F_2-Isoprostane Metabolite Assay 21049

Immunoassay for F_2-Isoprostane Metabolite in Urine

For Research Use Only, Not Intended For Use in Diagnostic Procedures

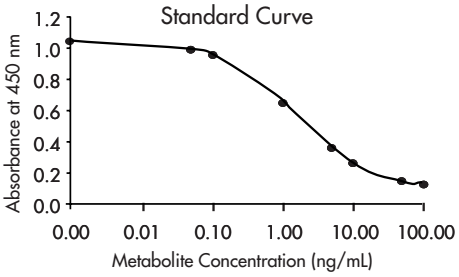
Catalog Number 21049

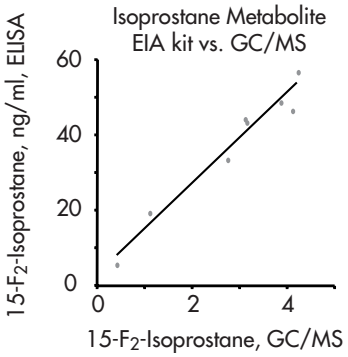
F_2-isoprostanes formed as a result of free radical-mediated non-enzymatic peroxidation of membrane-bound arachidonic acid are largely metabolized before excretion. They can be found in esterfied tissues, plasma lipids and other body fluids (1). Thus, these isoprostanes can be used to evaluate local or systemic lipid peroxidation *in vivo*. Four classes have been described, each potentially present in 16 isomers. 8-epi-prostaglandin- $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$ or sometimes called iP $F_{2\alpha}$ -III), a major component of the F_2-isoprostane family with mitogenic and vasoconstrictor capability, can be noninvasively measured in urine to assess *in vivo* lipid peroxidation (2). In humans, increased F_2-isoprostane levels have been found in different physiopathological conditions such as atherothrombotic disease, diabetes, hypercholesterolemia, Alzheimer's disease and cigarette smoking.

However, not all F_2-isoprostanes detected in urine may be of systemic lipid peroxidation origin. Three major urinary and plasma metabolites of 8-epi-PG $F_{2\alpha}$ have been reported in humans and rats: 2,3-dinor-5,6-dihydro-8-epi-PG $F_{2\alpha}$, 2,3-dinor-8-epi-PG $F_{2\alpha}$ and 2,3,4,5-tetranor-15-keto-13,14-dihydro-8-epi-PG $F_{2\alpha}$. In addition, autoxidation of α-linolenic acid found in plants will also produce 2,3-dinor-5,6-dihydro-8-epi-PG $F_{2\alpha}$, a major urinary metabolite in humans. Thus, the measurement of major metabolites of endogenous 8-epi-PG $F_{2\alpha}$ in addition to the parent compound may be useful for both (a) allowing the researcher to obtain a more accurate evaluation of the overall production of the biomarker *in vivo* while adding significance to individual measurements; and (b) provide a compound that can be measured without the risk of artifactual production *ex vivo*. This assay may be used for the quantification of metabolites of 8-epi-PG $F_{2\alpha}$ in samples without the need for prior purification or extraction.

F_2-Isoprostane Metabolite Method

The Bioxytech® F_2-Isoprostane Metabolite Assay is a competitive enzyme-linked immunoassay (ELISA) for determining levels of F_2-Isoprostane Metabolite. Briefly, the samples are mixed with a pre-treatment reagent. The F_2-Isoprostane Metabolite in the sample or standard then competes with F_2-Isoprostane Metabolite conjugated to horseradish peroxidase (HRP Conjugate) for binding to a polyclonal antibody specific for F_2-Isoprostane Metabolite coated on the microplate. Following substrate addition, the intensity of the color is proportional to the amount of F_2-Isoprostane Metabolite HRP Conjugate bound and inversely proportional to the amount of unconjugated F_2-Isoprostane Metabolite in the sample or standard.





The Bioxytech® Urinary F_2-Isoprostane Metabolite EIA is strongly recommended for use in urine and does not require solid phase extraction prior to sample assay. For measurement of the 8-isoprostane parent compound, please refer to our products 21019 and 21048.

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21049

F_2-Isoprostane Metabolite Product Summary

Intended Use:	Quantitative measurement of F_2-Isoprostane Metabolite. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	96 test colorimetric (ELISA)
Kit Contents:	• 96 well anti-F_2-Isoprostane Metabolite Coated Microtiter Plate • F_2-Isoprostane Metabolite Standard • Pretreatment Reagent • Wash Buffer • Dilution Buffer • TMB Substrate • HRP Conjugate
Storage and Stability:	12 months from date of manufacture when stored as specified.
Specimen Requirements:	Urine
Specificity:	Cross-reactivity at 50% B/B_0

F_2-Isoprostane Metabolite	100%
F_2-Isoprostane (8-epi-Prostaglandin $F_{2\alpha}$)	0.98%
Prostaglandin $F_{1\alpha}$	0.75%
11β-Prostaglandin $F_{2\alpha}$	0.29%
6-Keto Prostaglandin $F_{1\alpha}$	< 0.25%
Thromboxane B_2	< 0.25%
Arachidonic Acid	< 0.25%

Sensitivity:	0.05ng/mL with best precision on steeper, linear portion of the curve above 0.1ng/mL.

Bioxytech® MPO-EIA Assay 21013

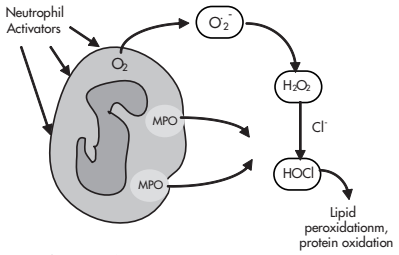
Immunoassay for Human Myeloperoxidase

For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 21013

Human myeloperoxidase (MPO) is a hemoprotein with a molecular weight of 140 kDa. It is composed of two heavy subunits of 53 kDa and of light subunits of 15 kDa. Each MPO molecule contains two prosthetic porphyrins, which play an essential role in the catalytic cycle.

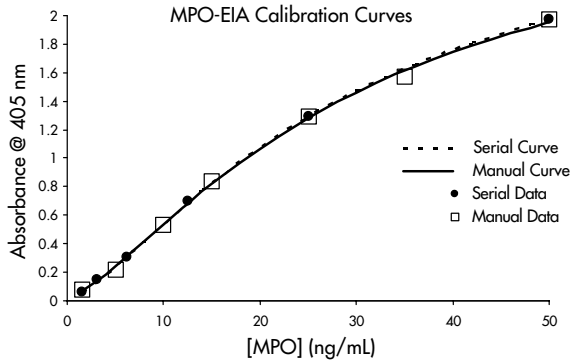
MPO is stored in primary granules (azurophilic) of neutrophils. It is a major component of the bactericidal armamentarium of neutrophils, due to its capacity to catalyze the production of hypochlorus acid (HOCl) a powerful oxidant. HOCl is derived from the chloride ion (Cl^-) and hydrogen peroxide (H_2O_2). MPO is a specific marker for polymorphonuclear cells (PMNs). It is released extracellularly via degranulation after PMN activation *in vitro or in vivo*. Extracellular MPO can be monitored as an index of PMN activation in a variety of clinical conditions where neutrophils contribute to inflammatory processes and subsequent disease pathology. These include rheumatoid arthritis, adult respiratory distress syndrome (ARDS), septic shock, psoriasis, gout, inflammatory bowel disease (IBD), emphysema and cardiovascular disease.



NEUTROPHIL

MPO-EIA Method

The Bioxytech® MPO-EIA assay system is a standard three (3) step "sandwich" ELISA. In step one, a plate bound (e.g. "solid phase") anti-human MPO monoclonal antibody captures sample and/or standard MPO. In step two, the bound MPO antigen is detected using a biotin-labeled polyclonal anti-human MPO. Finally, step three involves binding of an avidin/alkaline phosphatase conjugate to the biotinylated antibody. p-nitophenyl phosphate (pNPP) is then added causing the enzymatic hydrolysis of pNPP. The formation of yellow *p*-nitrophenol is monitored at 405 nm and the amount of sample MPO is calculated against the color development of on-board standards.



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21013

MPO-EIA Product Summary

Intended Use:	Quantitative measurement of human myeloperoxidase. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	96 Test Colorimetric (ELISA)
Kit Contents:	• 96 Anti-Hu-MPO Coated Microtiter Wells (6x16 strips) plus frame • Purified, Lyophilized MPO Standard • Biotin-Coupled Polyclonal Anti-MPO • Avidin-Coupled Alkaline Phosphatase Solution • pNPP Tablets Plus Diluting Buffer • Stop Solution • Diluting and Wash Buffers
Storage and Stability:	12 months from date of manufacture when stored as specified.
Specimen Requirements:	Human samples: serum, plasma, urine, bronchoalveolar lavage (BAL), cerebrospinal fluid (CSF), and cell culture supernatant.
Specificity:	Less than 2% cross reactivity with eosinophil peroxidase.
Sensitivity:	Threshold detection limit of 1.5 ng/mL

MPO-EIA Selected Research Applications

The Bioxytech® MPO-EIA has been used in researching various conditions including cadiovascular, fertility, pulmonary, RBC abnormalities, toxicity, inflammatory bowel disease, aging, "critically ill patient status", and transplantation.

Under certain circumstances, an excess of the reactive species produced by MPO (e.g., halides) can overwhelm local defenses and lead to oxidative stress and tissue injury. A process implicated in this pathogenesis is athrosclerosis where MPO is one pathway leading to protein and lipoprotein oxidation. Using OXIS' MPO-EIA kit, Bekesi's (1) showed that MPO could be used as an indicator of age and gender dependent cardiovascular risk. Menopause is positively correlated with decreased particle size following increased oxidation of LDL leading to cardiovascular disease. Hermenegildo (2) studied the effect of MPO level in the plasma of estradiol (E2) treated women. They found that MPO levels might undergo "bimodal function" where low dose E2 treatment promotes decreased levels of MPO (beneficial) and high dose E2 increases MPO levels. Altered functions of neutrophils have been demonstrated in blood from asthmatic patients both before and after challenge with antigens. For example, plant pollen (allergen) sensitized asthmatics were found to exhibit significantly higher levels of MPO released by neutrophils than non-sensitized controls (3). Red blood cell and platelet abnormalities cause essential thrombocythemia (ET) and polycythemia vera (PV). The Falanga group showed that MPO levels in white blood cells of ET and PV patients were significantly increased compared to controls (4). In a toxicity study, Alexis et al. (5) demonstrated that constituitive airway CD-14 (primary receptor for inhaled endotoxin such as lipopolysaccharide (LPS)) expression can predict the magnitude of post LPS inhalation neutrophil response. The authors showed increased CD-14 expression results in increased levels of MPO (5). Increased MPO production has also been reported in patients with inflammatory bowel syndrome. Using a rat model, Pacheco et al. (6) showed at corticoid treatments (budesonide and prednisone) significantly reduced the level of MPO, compared to the control subjects.

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6. Corticosteroid pretreatment prevents small intestinal mucosal lesion induced by acetic acid-perfusion model in rats. Pacheco I et al. (2000). Dig. Diseases and Sciences 45: 2337-46

Bioxytech® Lactof-EIA Assay 21015

Immunoassay for Human Lactoferrin

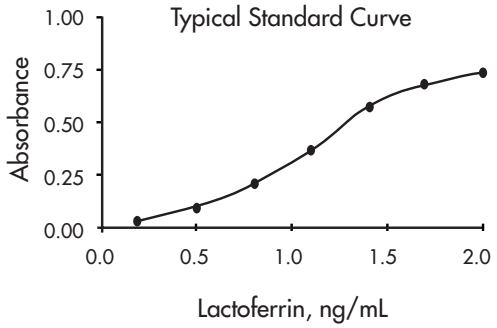
For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 21015

Human lactoferrin (LTF) is an 80 kDa glycoprotein which was first isolated from human milk. LTF is found in most of the body fluids and secretions, in the nose, genital tract and tear (Autiero, 1991). LTF in blood is secreted from the neutrophils and its plasma concentration is positively related to the total pool of neutrophils and to the rate of neutrophil turnover (Birgens, 1985). Because of its ability to strongly bind iron, LTF is considered to be bactericidal (Baynes, 1986). In a number of cases of inflammation, lactoferrin is released from secondary granules of neutrophilic leukocytes into the extracellular medium (Lash, 1983; Kälhler, 1988). Its extracellular concentration can therefore be used as an index of neutrophil activation, especially in blood samples containing anti-myeloperoxidase antibodies.

Lactof-EIA Method

The Lactof-EIA method is an enzyme-linked immunosorbent assay (ELISA). Samples are incubated in the wells of a sectionable microplate which have been coated with a monoclonal antibody to LTF. The LTF-MAb complex is detected by a biotinylated-monoclonal antibody to LTF. The final step of the assay is an amplification based on a biotin-avidin coupling in which avidin has been covalently linked to horse radish peroxidase. The amount of LTF is measured enzymatically upon addition of ortho-phenylenediamine (OPD) at 420 nm.



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2. Birgens HS (1985) "Lactoferrin in plasma measured by an ELISA technique: Evidence that plasma lactoferrin is an indicator of neutrophil turnover and bone marrow activity in acute leukemia" *Scand. J. Haematol*. 34: 326-331.

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4. Lash JA, Coates TD, Lafuze J, Baehner RL, Boxer L (1983) "Plasma lactoferrin reflects granulocyte activation *in vivo*" *Blood*. 61: 885-888.

5. Kähler S, Christophers E,. Schröder JM (1988) "Plasma lactoferrin reflects neutrophil activation in psoriasis" *Brit. J. Derm*. 119: 289-293.

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21015

Lactof-EIA Product Summary

Intended Use:	Quantitative measurement of human lactoferrin. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	96 test colorimetric (ELISA)
Kit Contents:	• 96 Anti-LTF Coated Microtiter Wells (6x16 strips) Plus Frame • Purified, Lyophilized LTF Standard • Biotin-Coupled Monoclonal Anti-LTF • Avidin-Coupled Alkaline Horseradish Peroxidase Solution • OPD Tablets Plus Diluting Buffer • Stop Solution • Diluting and Washing Buffers
Storage and Stability:	12 months from date of manufacture when stored as specified.
Specimen Requirements:	Human samples: serum, EDTA plasma, urine, bronchoalveolar lavage (BAL), cerebrospinal fluid (CSF), and cell culture supernatant.
Sensitivity:	Threshold detection limit of 1.0 ng/mL

Lactof-EIA Selected Research Applications

The OXIS LTF-EIA assay has been used to investigate subjects as diverse as pregnancy, exercise and immunology including Aids among others.

Pancora et al. studied the concentrations of lactoferrin with gestational age, infection, labor and rupture. Human amniotic fluid and plasma were evaluated for lactoferrin content using the OXIS LTf-EIA kit (1).

Santos-Silva et. al. studied oxidative stress in highly competitive swimmers (20 hrs/week) versus moderately exercising (1-2 hr/week) adeolescents (12 to 16 yrs) in terms of leukocyte activation and lipid profile. The authors reported elevated lactoferrin, elastase and GM-CSF in competitive swimmers, though only elastase presented a statistically significant difference (2). Note: OXIS now has an α_1-antiproteinase kit (colorimetric assay). α_1AP-410 can be used to indirectly evaluate the function of elastase activity. When α_1-antiproteinase is inactivaated (as a result of oxidative stress-protein oxidation) elastase will be higher.

Da Silva et al. studied the effect of the antibody CD11a mAb (ODULIMONAB) in preventing renal ischemia-reperfusion injury during kidney translpantation. A primate model was used wherein histological studies in kidney and creatinine clearance did not provide good indicators for glomerular function/neutrophil infiltration. Lactoferrin, however, did provide a good marker for neutrophil infiltratio/retention. It was significantly higher in untreated animals than the ODULIMONAB treated ones (3).

In other studies, Dapsanse et al. showed that plasma levels of LTF in HIV 1-infected ("AIDS") patients was decreased in correlation with the progression of the disease (4). This profile was not found in HIV-2 infected patients (HIV-2 progression is far slower than HIV-1). In parallel with the drop in LTF, a marked increase in circulating nitrogen derivatives (total nitrites) was observed in HIV-1 infected patients whereas a low level was found in normal donors and in HIV-2 infected patients. Thus, they suggest that impairment of circulating LTF could be an important element in HIV- induced oxidative imbalance.

1. Lactoferrin in intrauterine infection, human parturition, and rupture of fetal membrane.
Pacora, P et al. (2000). Am. J. Obstetrics and Gynecology 283: 904-10

2. Leukocyte activation, erythrocyte damage, lipid profile and oxidative stress imposed by high competition physical exercise in adolescents.
Santos-Silva A et al. (2001). Clinica Chimica Acta 306:119-126

3. A primate model of renal ischemia-reperfusion injury for pre-clinical evaluation of the anti-luekocyte function associated antigen 1 monoclonal antibody ODULIMONAB
Da Silva, M et al. (2001). J. Urology. 168:1915-1919

4. Differential pattern in circulating nitrogen derivatives, lactoferrin, and anti-lactoferrin antibodies in HIV Type 1 and HIV Type 2 infection.
Dapsanse V et al. (2001) AIDS Research and Retroviruses 17(11): 1041-45

Bioxytech® NFκB Chemiluminescent Assay 21050

Oligonucleotide / Immunoassay for NFκB

For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 21050

NFκB belongs to a family of nuclear transcription factors that are activated by various stress related stimuli such as growth factors, cytokines, UV light, pharmacologic and other stress associated factors. NFκB is comprised of subunits that form dimers each having various degrees of affinity for target genes. The best characterized of the NFκB transcription factors is the p65(RelA)-p50 dimer.

NFκB activation involves the removal of IκB, an inhibitor associated with the two major subunits p65(RelA) and p50 of the "inactive" NFκB. Removal of IκB allows translocation of the "active" NFκB (p65-p50 complex) to the nucleus where it then promotes transcription of specific genes by binding to a concensus sequence known as the kappa B motif (5'-GGGACTTTCC-3'). This sequence can be found in the enhancer region of genes involved with immune, inflammatory and acute phase stress response to oxidative or other challenges. Simultaneous up regulation of these genes results in a well orchestrated response to a threat to the health of a cell or organism. The NFκB motif can also be found in genes linked to cancer, viral replication and apoptosis.

NFκB Chemiluminescent Method

The Bioxytech® NFκB Chemiluminescent Assay is a "sandwich" type ELISA kit. An oligonucleotide containing the kappa B motif is bound to a 96 well plate. Sample or Standard NFκB added to the plate will specifically bind to the oligonucleotide. The DNA/NFκB is then recognized by a primary antibody that is specific for both p50 and p105 NFκB subunits. Finally, a secondary alkaline phosphatase antibody conjugate is added followed by a substrate allowing for detection of chemiluminescence and quantification in terms of Relative Light Units.

The Bioxytech® method allows for sensitive detection for either activated (nuclear) or total (cytosolic + nuclear) forms of NFkB as exemplified in the figures below.

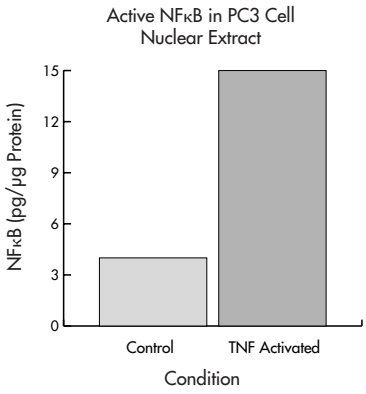

Figure 1 shows an increase in "active" NFkB found in the nuclear extract of a human prostate cancer cell line.

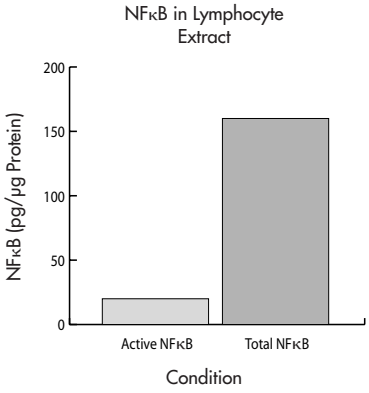

Figure 2 compares "active" NFkB measured from lymphocyte nuclear extract with "total" NFkB measured from whole cell lysate.

References

1. Hattori Y, et al. (2000). High glucose-induced nuclear factor- activation in vascular smooth muscles. Cardiovascular Research 46: 1888-197.
2. Davis JN, et al. (2001). Soy isoflavone supplementation in healthy men prevents NF-κB activation by TNF-α in blood lymphocytes. Free Rad. Biol. Med. 30(11): 1293-1302.
3. Colantoni A, et al. (2000). Dose-dependent effect of ethanol of hepatic oxidative stress and interluukin-6 production after burn injury in the mouse. Alcohol Clin. Exp. Res. 24(9): 14348-48.
4. Gilmore TD, et al. (1996). Rel/ NF-κB/ I-κB proteins and cancer. Oncogene 13: 1367-78.
5. Thanos D, Maniatis T (1995). NF-κB: a lesson in family values. Cell 80: 529-32.

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21050

NFκB Chemiluminescent Product Summary

Intended Use:	Quantitative measurement of NFκB in biological samples. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	96 Well chemiluminescent
Kit Contents:	• Oligonucleotide Coated Plate • NFκB Standard • Dilution Buffer • Activated NFκB Binding Buffer • Total NFkB Binding Buffer • Wash Buffer • Non-Specific DNA • 1° P50, P105 Specific Antibody • 2° Alkaline Phosphatase Antibody • Conjugate • Enzyme Substrate • Reagent Trough
Storage and Stability:	12 months from date of manufacture when stored as specified.
Specimen Requirements:	Cell and tissue lysates or nuclear extracts
Assay Precision:	Intra-assay (%CV) <5.0 Inter-assay (%CV) <10.0
Sensitivity:	10 pg NFκB

Bioxytech® Nitrotyrosine-EIA Assay 21055

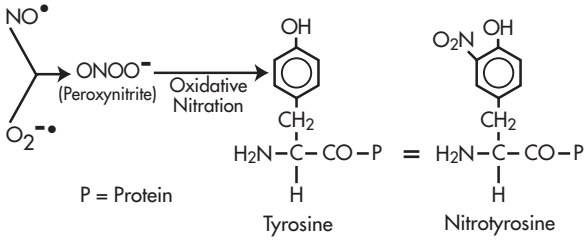
Quantitative Measurement of Nitrotyrosine

For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 21055

Nitrotyrosine is a relatively stable product formed from various reaction pathways. Perhaps most notable is the reaction of peroxynitrite (formed from superoxide and nitric oxide radicals) with protein bound tyrosine.

As a strong oxidant and nitrating agent, peroxynitrite mediates tyrosine nitration reactions on proteins resulting in inactivation of certain housekeeping enzymes (e.g. α_1-antiproteinase) as well as endogenous antioxidant enzymes such as catalase and SOD.



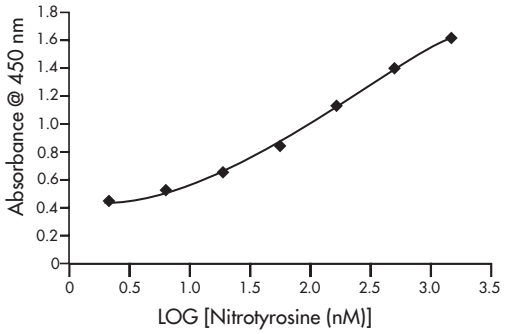
This form of protein nitration has been shown to be involved in the pathology of several human conditions such as chronic myocardial dysfunction, respiratory distress syndrome, inflammatory bowel disease, lung injury, asthma, atherosclerosis, rheumatoid arthritis, chronic renal failure, ALS, septic shock and others.

Nitrotyrosine EIA Method

The Nitrotyrosine-EIA is a "sandwich" ELISA. Antigen captured by a solid phase monoclonal antibody is detected with a biotin-labeled goat polyclonal anti-nitrotyrosine. An streptavidin peroxidase conjugate then binds to the biotinylated antibody. The tetramethylbenzidine (TMB) substrate is added and the yellow product is monitored at 450 nm.

Demonstration of nitrotyrosine in biological samples is useful in determining oxidative stress level and specifically infers the presence of peroxynitrite or related nitrogen-centered oxidants. The new Nitrotyrosine ELISA kit from *Oxis*Research® provides a simple and reproducible method for NT detection in tissues and plasma.

Typical NT-EIA Standard Curve



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2. Wu W, et al. (1999) "Eosinophil peroxidase nitrates protein tyrosyl residues" J. Biol. Chem. 274(36):25933-44.
3. van der Vliet A, et al. (1999) "Reactive nitrogen species and tyrosine nitration in the respiratory tract: Epiphenomena or a pathobiological mechanism of disease" *Am. J. Respir. Cri. Care. Med.* 160: 1-9.
4. Whiteman M., et al. (1996) "Protection against peroxynitrite-dependent tyrosine nitration and á-antiproteinase inactivation by oxidized and reduced lipoic acid" *FEBS Lett.* 379: 74-6.
5. Keng T., et al. (2000) "Peroxynitrite formation and decreased catalase activity in autoimmune MRL-lpr/lpr mice" *Mol. Med.* 6(9): 779-92.
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8. Kooy NW, et al. (1997) "Extensive tyrosine nitration in human myocardial inflammation: evidence for the presence of peroxynitrite" *Crit. Care Med.* 25(5): 812-9.
9. Beal MF, et al. (1997) "Increased 3-nitrotyrosine in both sporadic and familial amyotrophic sclerosis" *Ann. Neurol.* 42: 646-54.
10. Sittipunt C, et al. (2001) "Nitric oxide and nitrotyrosine in the lungs of patients with acute respiratory distress syndrome" *Am. J. Res. Crit. Care Med.* 163: 503-510.
11. Singer II, et al. (1996) "Expression of inducible nitric oxide and nitrotyrosine in colonic epithelium in inflammatory bowel disease" *Gastroenterology* 111: 8771-85.
12. Chang L-Y, Crapo JM, (2002) "Inhibition of airway inflammation and hyper-reactivity by an antioxidant mimetic" *Free Radic. Biol. Med.* 33(3): 379-85. (Additional references available upon request)

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21055

Nitrotyrosine EIA Product Summary

Intended Use:	Quantitative measurement of nitrotyrosine in biological samples. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	2 X 96 well plates
Kit Contents:	• Sample Diluting Buffer • Nitrotyrosine Standard • Nitrotyrosine Antibody • Streptavidin Peroxidase • TMB Substrate • Substrate Buffer • Substrate Diluting Buffer • Wash Buffer • Stop Solution • Pre-Coated Plates w/ Frame
Storage and Stability:	12 months from date of manufacture when stored as specified.
Specimen Requirements:	Tissue, plasma
Assay Precision:	
Intra-assay (%CV)	2.32
Inter-assay (%CV)	11.17
Sensitivity:	LLD = 2 nM

Bioxytech® Nitric Oxide Assay (enzymatic) 22110

Quantitative Measurement of Total Nitrite in Aqueous Samples

For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 22110

Nitric Oxide (NO; also known as endothelial derived relaxation factor or EDRF) is produced in trace quantities in neurons, endothelial cells, platelets and neutrophils in response to homeostatic stimuli. Under these conditions, NO acts in a paracrine fashion to transduce cellular signals. NO is also produced by other cell types (macrophages, fibroblasts, hepatocytes) in micromolar concentrations as a response to inflammatory or mitogenic stimuli. In this case, the biological role is defense against pathogens through oxidative toxicity. NO reacts readily with other radicals, metal ions and protein thiols resulting in a short biological half-life. High NO levels can lead to the formation of peroxynitrite (ONOO), destruction of iron-sulfur clusters, thiol nitrosation and nitration of protein tyrosine residues.

The Bioxytech® NO Enzymatic assay provides reagents for use in the determination of nitrite as an indicator of nitric oxide production in biological samples. Nitric oxide is rapidly converted to nitrite and nitrate in typical oxygenated aqueous solutions. Because an excellent colorimetric reagent (the Griess reagent) exists for the determination of nitrite, it is common practice to use enzymatic or chemical reduction to convert all nitrate to nitrite in a sample and measure total nitrite as an indicator of nitric oxide production. This assay provides for enzymatic reduction of nitrate by nitrate reductase, followed by spectrophotometric analysis of total nitrite using Griess reagent. In addition to providing all necessary components in a microtiter format, this kit employs affinity purified Zea mays nitrate reductase and NADH, thereby circumventing some of the potential problems reported for nitrite measurement using NADPH dependent nitrate reductases.

NO-Enzymatic Method

In aqueous solution, nitric oxide rapidly degrades to nitrate and nitrite (Figure 1). Spectrophotometric quantitation of nitrite using Griess Reagent is straightforward and sensitive, but does not measure nitrate, causing a possible underestimation of nitric oxide. This kit employs the NADH-dependent enzyme nitrate reductase for enzymatic reduction of nitrate to nitrite (Figure 1) prior to quantitation of nitrite using Griess reagent. In acid solution, nitrite is converted to nitrous acid (HNO_2) which diazotizes sulfanilamide. This sulfanilamide-diazonium salt is then reacted with N-(1-Naphthyl)-ethylenediamine (NED) to produce a chromophore which is measured at 540 nm.

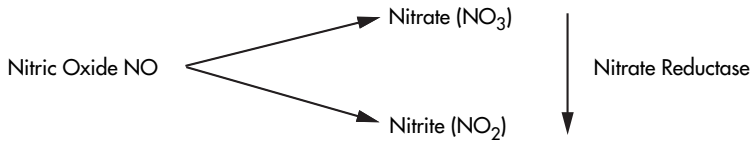

Figure 1 - Enzymatic Reduction of Nitrate to Nitrite

This kit can be used to accurately measure as little as 1 µM of nitrite (final concentration in the assay). Very little sample is required (5 µL to 85 µL for most samples) so it is the assay we recommend for testing of samples such as rodent tail bleeds etc. For larger samples we recommend our NO-540 (22116) cadmium reductant assay.

References

1. Schmit, H.H., et. al. Biochemica 2:22-23 (1995).

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22110

NO-Enzymatic Product Summary

Intended Use:	Quantitative measurement of total nitrite in aqueous samples. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	96 Test Microplate
Kit Contents:	• Nitrate Reductase • NADH • Color Reagent #1 (Sulfanilamide) • Color Reagent #2 (N-(1-Naphthyl) ethylenediamine dihydrochloride) • Nitrate Standard • Microtitre Plate
Storage and Stability:	12 months from date of manufacture when stored as specified.
Specimen Requirements:	Tissue homogenates, plasma, cell lysate and culture supernatant .
Sensitivity:	1.0 µM

NO-Enzymatic Selected Research Applications

The authors studied circadian rhythms in urinary MDA, urinary 8-Isoprostane, urinary 8-OHdG and serum's total nitrite and uric acid in diabetic (4) and "healthy" men (7), using NO-22110, 8-OHdG-EIA, and 8-Isoprostane-ELISA.

1. Circadian variation in oxidative stress markers in healthy and type II diabetic men.
Kanabrocki, E.L., et. al., (2002) Chronobiology International 19(2):423-39

Bioxytech® Nitric Oxide Assay (non-enzymatic) 22111

Quantitative Measurement of Total Nitrite in Aqueous Samples

For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 22111

Nitric Oxide (NO; also known as endothelial derived relaxation factor or EDRF) is produced in trcae quantities in neurons, endothelial cells, platelettes and neutrophils in response to homeostatic stimuli. Under these conditions, NO acts in a paracrine fashion to transduce cellular signals. NO is also produced by other cell types (macrophages, fibroblasts, hepatocytes) in micromaolar concentrations as a response to inflammatory or mitogenic stimuli. In this case, the biological role is as a defense against pathogens through oxidative toxicity. NO reacts readily with other radicals, metal ions and protein thiols resulting in a short biological half-life. High NO levels can lead to the formation of peroxynitrite (ONOO), destruction of iron-sulfur clusters, thiol nitrosation and nitration of protein tyrosine residues.

The Bioxytech® NO-Non-Enzymatic assay provides reagents for use in the determination of nitrite as an indicator of nitric oxide production in biological samples. Nitric oxide is rapidly converted to nitrite and nitrate in typical oxygenated aqueous solutions. Because an excellent colorimetric reagent (the Griess reagent) exists for the determination of nitrite, it is common practice to use enzymatic or chemical reduction to convert all nitrate to nitrite in a sample and measure total nitrite as an indicator of nitric oxide production. This assay provides for chemical reduction of nitrate by granulated cadmium, followed by spectrophotometric analysis of total nitrite using Griess reagent. In addition to providing all necessary components in a microtiter format, this kit provides for elimination of possible interference by proteins in concentrated biological samples by $ZnSO_4$ precipitation. The granulated cadmium beads provide for a quantitative and inexpensive assay.

NO-Non-Enzymatic Method

In aqueous solution, nitric oxide rapidly degrades to nitrate and nitrite (Figure 1). Spectrophotometric determination of nitrite using Griess reagent is straight forward and sensitive, but does not measure nitrate, causing a possible underestimation of nitric oxide. This kit employs granular cadmium metal for chemical reduction of nitrate to nitrite (Figure 1) prior to quantitation of nitrite using Griess reagent. In acid solution, nitrite is converted to nitrous acid (HNO_2) which diazotizes sulfanilamide. This sulfanilamide-diazonium salt is then reacted with N-(1-Naphthyl)-ethylenediamine (NED) to produce a chromophore which is measured at 540 nm.

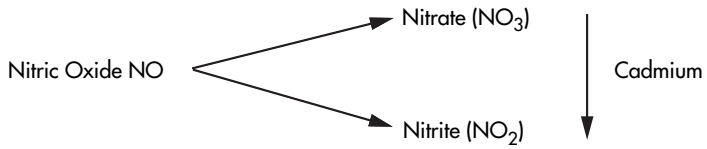

Figure 1 - Chemical Reduction of Nitrate to Nitrite

This kit can be used to accurately measure as little as 0.25 µM of nitrite (final concentration in the assay). Very little sample is required (10 µL to 100 µL for most samples). For faster reduction time we recommend our NO-540 (22116) another cadmium reductant assay. The NO-540 employs a cadmium powder to effectively increase surface area to sample ratio thereby drastically reducing sample reduction time.

References

1. Schmidt, H.H., et. al. Biochemica 2:22-23 (1995).

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22111

NO-Non-Enzymatic Product Summary

Intended Use:	Quantitative measurement of total nitrite in aqueous samples. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	96 test microplate
Kit Contents:	• Granulated Cadmium (25g Cd beads) • $ZnSO_4$ Solution (30% wt/vol) • Microcentrifuge Tubes (50 X 1mL) • Color Reagent #1 (Sulfanilamide) • Color Reagent #2 (N-(1-Naphthyl) ethylenediamine dihydrochloride) • Nitrite standard • Microtitre Plate • Cadmium Bead Washing Solution
Storage and Stability:	12 months from date of manufacture when stored as specified.
Specimen Requirements:	Tissue homogenates, plasma, cell lysate and culture supernatant .
Sensitivity:	0.25 µM

NO-Non-Enzymatic Selected Research Applications

Hyperglycemia is linked to vascular and glomerular dysfunction by many mechanisms, including a glucose-dependent abnormality in nitric oxide production and action. The authors investigated NO biosynthesis and action associated with glomerualr infiltration and persistent micro albuminnuria in type 1 diabetes patients.

1. Increased circulating nitric oxide in young patients with type 1 diabetes and persistent micro albuminuria: relation to glomerular hyperfiltration.
Chiarelli F et al., (2000). Diabetes 49: 1258-63.

Bioxytech® Nitric Oxide Synthase Assay (radioactive arginine) 22112

Quantitative Assay for the Determination of NOS Activity

For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 22112

Three distinct isoforms of NOS have been identified: inducible (iNOS), endothelial (eNOS) and neuronal (nNOS or bNOS). Nitric Oxide Synthase is a remarkably complex enzyme that acts on molecular oxygen and arginine in neurons, endothelial cells, platelets, neutrophils and other cells to produce nitric oxide (NO). Nitric oxide is a unique second messenger molecule that readily diffuses through cell membranes to exert a variety of biological actions in mammalian cells. Excess generation of NO can lead to the formation of peroxynitrite, destruction of iron-sulfur clusters, thiol nitrosylation and nitration of protein tyrosine residues.

Measuring NOS activity by monitoring the conversion of arginine to citrulline is a widely accepted assay for NOS activity in both crude and purified enzyme preparations. Advantages of the NOS assay kit include the use of radioactive substrates (^{3}H-arginine or ^{14}C-arginine), and an easy method for separating neutrally charged citrulline from positively charged arginine.

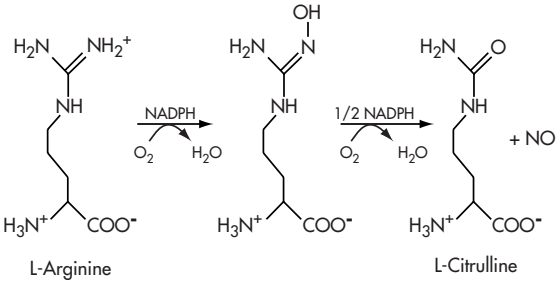
The citrulline assay has been used to quantify levels of NOS activity in tissue homogenates from numerous sources including blood vessels, immune cells, visceral organs, nervous tissue and cultured cells. Nitric oxide synthase is relatively unstable; therefore, tissues should be harvested quickly after animal euthanasia. If enzyme assays are to be performed at a later time, it is best to freeze intact tissues or harvested cultured cells prior to homogenization. Wrap the tissues in aluminum foil, flash freeze in liquid nitrogen and then store at -70°C.

The subcellular distribution of NOS is tightly regulated in tissues. Endothelial NOS is largely membrane associated as a result of N-terminal myristoylation. bNOS is found primarily in the cytoplasmic fractions in adult rat brain, yet in skeletal muscle, it is predominately associated with membrane fractions. Nitric oxide synthase in soluble and membrane-associated fractions can be separated by centrifuging the homogenized tissues at 100,000 x g for 60 minutes. The supernatant contains soluble NOS, while the pellet, which can be re-suspended in homogenization buffer, will contain membrane-associated NOS.

NOS Arginine Method

This NOS assay kit is based on the biochemical conversion of L-arginine to L-citrulline by NOS. This reaction involves a five-electron oxidation of a guanidinonitrogen of L-arginine to nitric oxide (NO), together with the stoichiometric production of L-citrulline. The reaction consumes 1.5 equivalents of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and also requires molecular oxygen, calcium, calmodulin and tetrahydrobiopterin.

For routine assays, radioactive arginine is added to tissue or cellular extracts. After incubation, the reactions are stopped with a buffer containing EDTA, which chelates the calcium required by NOS and, consequently, inactivates the NOS. Equilibrated resin, which binds to arginine, is added to the samples and they are centrifuged in spin cups. The citrulline, being ionically neutral at pH 5.5, passes through the spin cup column. The NOS activity is then reported by quantitating the radioactivity in the eluate. Nitric oxide synthase activity in the citrulline assay is defined as counts per minute (cpm) in an incubated test sample as compared to an appropriate blank.



NOS catalyzes a 5-electron oxidation of an amidine nitrogen of L-arginine to generate NO and L-ctrulline. L-Hydroxyarginine is formed as an intermediate that is tightly bound to the enzyme. Both steps in the reaction are calcium and calmodulin dependent.

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22112

NOS Arginine Product Summary

Intended Use:	Determination of NOS activity by monitoring the conversion of tritiated L-arginine to L-Citrulline. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	50 tests
Kit Contents:	Part 1, room temperature storage - • Homogenization Buffer • Stop Buffer (stops cNOS reactions) • Equilibrated Arginine-Binding Resin • Calcium Chloride ($CaCl_2$) • Elution Buffer • Spin Cups and Cup Holders Part 2, -70°C storage - • Cerebellum Extract • Calmodulin • Reaction Buffer • NADPH Tetrasodium Salt • N^G -Nitro-L-arginine Methyl Ester, HCl, (L-NAME, HCl),
Storage and Stability:	2 years from date of manufacture when stored as specified.
Specimen Requirements:	Tissue homogenates and cell lysates.

NOS Arginine Selected Research Applications

Role of nitric oxide relative to gastric mucosal injury is examined. Nitric oxide appears to play a protective role in the gastric mucosa as an endogenous vasodilator and its hyperemic response to damaging agents. The authors used NO modulators (L-arginine and NG-nitro-L-aginine methyl ester or L-NAME) in rats and measured the NOS activity of gastric tissue using the OXIS NOS 22112 kit (1).

Nishima et al evaluated the effect of a new NOS inhibitor in a hamster model of diaphragmatic dysfunction. Hamster diaphragmatic muscle was tested for nitric oxide using the OXIS NO assay (22112).

Propofol, a peroxynitrite scavenger and antioxidant, is often used in critically ill patients as a sedative or anesthetic. A potential negative effect of the antioxidant activity of this product includes increased severity of sepsis. Mikawa et al. investigate whether propofol may suppress the lipid peroxidation and NO species formation. They evaluated the effect of a new iNOS inhibitor on a rat model of pancreatitis wherein diaphragmatic muscle homogenate was tested for nitric oxide using the OXIS NO assay (22112).

1. Role of nitric oxide and mucus in ischemia/reperfusion-induced gastric mucosal injury in rats.
Kim, H et al. (2001). Pharmacology 62: 200-207.

2. ONO1714, a new inducible nitric oxide synthase inhibitor, attenuates sepsis-induced diaphragmatic dysfunction in hamsters.
Nishima K, et al. (2001). Anesthesia and analgesia 92: 959-66.

3. ONO-1714, a new inducible nitric oxide synthase inhibitor, attenuated diaphragmatic dysfunction associated with cerulein-induced pancreatisits in rats.
Mikawa K et al. (2001). Crit. Care Med. 29(6) 1215-21.

Bioxytech® Nitric Oxide Synthase Assay (colorimetric) 22113

Colorimetric Assay for the Measurement of NOS Activity

For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number 22113

Three distinct isoforms of NOS have been identified: inducible (iNOS), endothelial (eNOS) and neuronal (nNOS or bNOS). Nitric Oxide Synthase is a remarkably complex enzyme that acts on molecular oxygen and arginine in neurons, endothelial cells, platelets, neutrophils and other cells to produce nitric oxide (NO). Nitric oxide is a unique second messenger molecule that readily diffuses through cell membranes to exert a variety of biological actions in mammalian cells. Excess generation of NO can lead to the formation of peroxynitrite, destruction of iron-sulfur clusters, thiol nitrosylation and nitration of protein tyrosine residues. The final products of NO *in vivo* are nitrate (NO_3^-) and nitrite (NO_2^-).

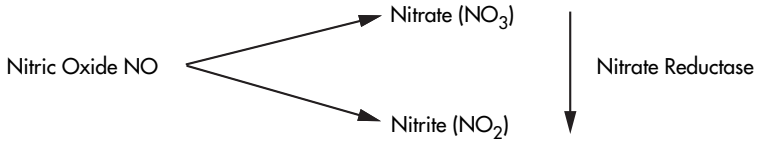
The Bioxytech® NOS Colorimetric Assay Kit is intended for the quantitative determination of total nitrite as an indicator of nitric oxide synthase (NOS) activity in biological samples. It may be used for the assay of total nitrite in urine, plasma, serum and tissue culture medium.

The subcellular distribution of NOS is tightly regulated in tissues. Endothelial NOS is largely membrane associated as a result of N-terminal myristoylation. nNOS is found primarily in the cytoplasmic fractions in adult rat brain, yet in skeletal muscle, it is predominately associated with membrane fractions. Nitric oxide synthase in soluble and membrane-associated fractions can be separated by centrifuging the homogenized tissues at 100,000 x g for 60 minutes. The supernatant contains soluble NOS, while the pellet, which is resuspended in homogenization buffer, contains membrane-associated NOS.

NOS-Colorimetric Method

NO exists primarily as NO_2 and NO_3 in biological systems. Its measurement can be a useful tool in estimating NOS activity. The principle of this assay is based on the measurement of NO_2^- produced in the sample during a timed reaction compared with a heat inactivated control sample. NOS mediated NO production is halted by heat inactivation. Subsequent comparison of heat inactivated with non heat inactivated samples, over time, yields an indication of NOS activity.

Nitrate reductase is utilized for the enzymatic reduction of nitrate to nitrite.



NADPH is an essential cofactor for the function of the NOS enzyme. Unfortunately, NADPH interferes with the Griess reagents, the most commonly used reagents for nitrite detection. There are two common methods to reduce the NADPH interference. Very small concentrations of NADPH can be used, in conjunction with a catalytic system for recycling of the spent $NADP^+$ back to NADPH; or the excess NADPH is removed or destroyed. This assay uses lactate dehydrogenase (LDH) to destroy the excess NADPH. Spectrophotometric quantitation of nitrite using Griess Reagents is straightforward and sensitive. In acidic solution, nitrite is converted to nitrous acid (HNO_2), which diazotizes sulfanilamide. This sulfanilamide-diazonium salt is then reacted with N-(1-Naphthyl)- ethylenediamine to produce a chromophore which is measured at 540 nm.

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22113

NOS-Colorimetric Product Summary

Intended Use:	Indirect determination of NOS activity by comparison of NO produced in active samples vs. heat inactivated samples. For Research Use Only. Not Intended For Use in Diagnostic Procedures.
Format:	80 microtitre tests
Kit Contents:	• Assay Buffer • Nitrate Reductase • Co-factor Preparation • Nitrate Standard • Lactate Dehydrogensae • Color Reagent #1 (Sulfanilamide) • Color Reagent #2 (N-(1-Naphthyl) Ethylenediamine Dihydrochloride) • NADPH Tetrasodium Salt • Microtitre Plate
Storage and Stability:	Seven months from date of manufacture when stored as specified.
Specimen Requirements:	Tissue homogenates and cell lysates.
Sensitivity:	5 nmol/mL

Bioxytech® NO-540 Assay 22116

Nitric Oxide Levels by Measurement of Total Nitrite (NO_2^-) Levels

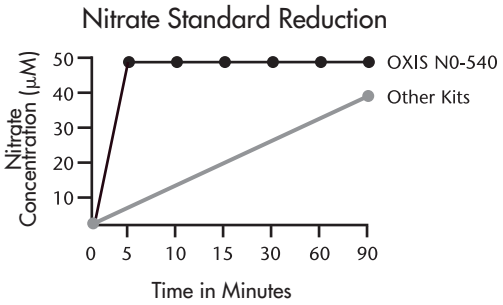
For Research Use Only, Not Intended For Use in Diagnostic Procedures

Catalog Number: 22116

Nitric oxide has been shown to function as a signal transmitter in organisms. While its EDRF (endothelial derived relaxation factor) activity is well described, it has also been recognized as cytotoxic, mutagenic, and carcinogenic. Because nitric oxide rapidly converts to nitrate and nitrite, sample reduction and measurement of nitrite has become commonplace in determining *invivo* NO production.

The improved NO-540 assay from *Oxis*Research® allows for simple determination of Nitric Oxide levels by measurement of Total Nitrite (NO_2^-) levels. The kit employs the popular Griess method simplified with same day cadmium reduction to maximize simplicity and reproducibility.

In tests designed to assess reduction rates of the various methods, the *Oxis*Research® assay was capable of fully reducing samples up to 100 µM in approximately 5 minutes. In contrast cadmium reduction as employed in existing kits requires overnight incubation. The improved reduction capacity of the NO-540 method is illustrated below.



NO-540 Method

Improvements over existing method:

- Convenient, pre-weighed cadmium for simple, timesaving sample preparation.
- Significantly improved sample reduction time.
- Micro-technique for small sample volume included.
- 96 well procedure included.

References

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NO-540 Product Summary

Intended Use: Quantitative measurement of total nitrite. For Research Use Only. Not Intended For Use in Diagnostic Procedures.

Summary of test: In biological samples, nitric oxide rapidly converts to nitrate and nitrite. A cadmium-based reduction of nitrate to nitrite is performed. Total nitrite is determined using Griess reagent.

Kit Contents:
- Sulfanilamide
- N-Naphthyl-Ethylenediamine
- 3x Assay Buffer
- Nitrite Standard
- Cadmium
- H_2SO_4
- $ZnSO_4$
- NaOH

Storage and Stability: 12 months from date of manufacture when stored at 4° C.

Specimen Requirements: Plasma, urine, cell lysates and tissue homogenates.

Assay Precision:

	Nitrate Control			Plasma
Plasma (µM)	1	10	100	26.2
Mean (A_{540} nm)	0. 02396	0.13661	1.12723	0.06409
Intra-assay precision (% CV)	6.13	1.186	0.66	1.71
Inter-day precision (% CV)	5. 82	0.22	0.13	0.45
Total precision (% CV)	7. 08	2.10	0.72	3.52

Sensitivity: 0.23 µM (in the assay)
2.3 µM in sample (pre-reduction)

NO-540 Selected Research Applications

LeVine et al. investigated surfactant effects during bacterial infection of the lung. Rat bronchoalveolar lavage (BAL) was evaluated for hydrogen peroxide and nitric oxide (NO) content using the OXIS H_2O_2-560 assay and the OXIS NO assay (1).

Oka et al. studied the effect of Royal Jelly (principal food source of the queen honey-bee produced by the hypopharingeal and mandibular glands of worker bees) on DNP antigen (Dinitro phenyl keyhole limpet hemocynin) induced mice. Mouse macrophage and mast cells (primary) from PECs (peritoneal exudate cells from the peritoneal cavity) were tested for GSH content using the OXIS GSH-400 kit. Cell culture fluid was tested for nitric oxide using an NO kit from OXIS (2)

1. Distinct effects of surfactant protein A or D deficiency during bacterial infection on the lung.
Le Vine, AM et al. (2000). J. Immunol. 165:3934-40.

2. Suppression of allergic reactions by royal jelly in association with the restoration of macrophage function and the improvement of Th1/Th2 cell responses
Oka H et al. (2001). Int. Immuno-pharmacology 193): 521-32

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