# **OXIDATIVE STRESS ASSAY KITS**



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# **Catalase Colorimetric & Fluorescent Activity Kits**

Colorimetric: K033-H1 (2 Plate) Fluorescent: K033-F1 (2 Plate)

## FEATURES

- Use Measure Catalase Activity in Any Sample
- Time to Answer 45 Minutes
- Sensitivity
  Measure as little as 0.052 U/mL
- Samples/Kit 89 in Duplicate
- Stability
  Liquid 4°C Stable Reagents
- ► Format 96-well
- Species
  Species Independent
- Readout
  Colorimetric: 560 nm
  - Fluorescent: 590 nm em/520 nm ex



## SCIENTIFIC RELEVANCE

Hydrogen peroxide,  $(H_2O_2)$  is one of the most frequently occurring reactive oxygen species. It is formed either in the environment, as a by-product of aerobic metabolism, superoxide formation and dismutation, or as a product of oxidase activity. Both excessive hydrogen peroxide and its decomposition product hydroxyl radical, are harmful for most cell components. Its rapid removal is essential for all aerobically living prokaryotic and eukaryotic cells.







One of the most efficient ways of removing peroxide is through the enzyme catalase, which is encoded by a single gene and is highly conserved among species. Mammals, including humans and mice, express catalase in all tissues. A high concentration of catalase can be found in the liver, kidneys and erythrocytes. The expression is regulated at transcription, post-transcription and posttranslation levels. High catalase activity is detected in peroxisomes.

Colorimetric

# **Ceruloplasmin (Cp) Colorimetric Activity Kit**

#### K035-H1 (2 Plate)

#### **FEATURES**

Use	Non-Invasive Pregnancy Marker
Sample	Urine, Serum
Validation	Humans, Felids, Polar Bear, Panda
Species	Multiple Species
Time to Answer	60 Minutes
Format	96-Well
Samples/Kit	89 in Duplicate
Stability	Liquid 4°C Stable Reagents
Readout	Colorimetric, 560 nm



#### SCIENTIFIC RELEVANCE

Ceruloplasmin (Cp) is an acute phase multicopper oxidase enzyme that normally plays a protective role in responses to immune-provoking stimuli and is also associated with reproduction. Estrogens alter the subcellular distribution of copper in the liver, leading to an increase in plasma copper levels and subsequent ceruloplasmin synthesis. Serum levels of Cp have been shown to increase during normal pregnancy in some species, possibly as a protection against the oxidative costs of reproduction. In giant pandas and some felids, urinary Cp activity has shown to be elevated in pregnant vs. pseudopregnant animals beginning in the first week of gestation and continuing throughout the luteal phase.





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# **DNA Damage ELISA Kits**

K059-H1 (1 Plate) | K059-H5 (5 Plate)

## **FEATURES**

Use	Measure oxidized guanine molecules	MULTI SPECIES	
Sample	Serum, Plasma, Saliva, Urine, Fecal Extracts, Digested DNA, TCM		
Time to Answer	2.5 hours		
Sensitivity	51 pg/mL		
Species	Species independent		
Samples/Kit	38 or 230 in Duplicate		
Readout	Colorimetric, 450 nm		

## SCIENTIFIC RELEVANCE

Free radicals and other reactive species are constantly generated in vivo and cause oxidative damage to biomolecules, a process held in check only by the existence of multiple antioxidant and repair systems as well as the replacement of damaged nucleic acids, proteins and lipids. Intracellular reactive oxygen species are produced as a result of normal metabolism and extracellular forms are produced as a result of ultraviolet or ionizing radiation. Cellular function may be interrupted or halted if DNA damage corrupts the integrity of essential information contained in the genome and it is widely thought that continuous oxidative damage to DNA is a significant contributor to the age-related development of major cancers. Among numerous types of oxidative DNA damage, the formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) is a ubiquitous marker of oxidative stress.



8-OHdG is physiologically formed and enhanced by chemical carcinogens. During the repair of damaged DNA *in vivo* by exonucleases the resulting 8-OHdG is excreted without further metabolism into urine, allowing for convenient detection.



# **Formaldehyde Fluorescent Detection Kit**

#### K001-F1 (2 Plate)

## **FEATURES**

Use	Measure Formaldehyde
Sample	Urine, Water, TCM
Convenient	No Extraction, No Chemical Derivatization
Samples/Kit	88 in Duplicate
Sensitivity	< 80 pM
Stability	Liquid 4° Stable Reagents
Time to Answer	30 Minutes
Readout	Fluorescent, 510 nm em/450 nm ex



#### SCIENTIFIC RELEVANCE

Formaldehyde (methanal), H<sub>2</sub>C=O, is a colorless, flammable, strong-smelling gas. It is an important industrial chemical used to manufacture building materials and to produce many household products. In the US approximately 3 x 10<sup>9</sup> Kg are produced annually. Formaldehyde is commonly used as an industrial fungicide, germicide, and disinfectant, and as a preservative in mortuaries and medical laboratories. Materials containing formaldehyde can release formaldehyde gas or vapor into the air. Formaldehyde can also be released by burning wood, kerosene, natural gas, or cigarettes, from automobile emissions, and from natural processes. Occupational exposure to formaldehyde by inhalation is mainly from three types of sources: thermal or chemical decomposition of



formaldehyde-based resins, formaldehyde emission from aqueous solutions (for example, embalming fluids), and the production of formaldehyde resulting from combustion. Formaldehyde can be toxic, allergenic, and carcinogenic. Because formaldehyde resins are used in many construction materials, it is one of the more common indoor air pollutants.



# **FRAP<sup>TM</sup> (Ferric Reducing Antioxidant Power) Detection Kit**

#### K043-H1 (2 Plate)

#### FEATURES

•	Use	Measure Ferric Reducing Anti-Oxidant Potential (FRAP) of Samples
•	Samples	Serum, Plasma, Tissue, Saliva, Cell Lysates, Urine, Food, Cosmetics, Additives
	Samples/Kit	89 in Duplicate
	Stability	Liquid 4°C Stable Reagents
	Time to Answer	30 Minutes
	Readout	Colorimetric, 560 nm



Potentially harmful reactive oxygen species (ROS) are produced as a consequence of normal aerobic metabolism. "Free Radicals" (FR) are usually removed or inactivated in vivo by a team of antioxidants. They are chemically stable atoms and molecules, which have one or more free electrons. Almost all biomolecules may be attacked by reactive free radicals. Free radicals are responsible for many pathological processes, or they can be generated as the result of the pathological stage and cause important secondary damage to biological systems and cells. Connections between free radicals and some serious diseases, including Parkinson's and Alzheimer's diseases, atherosclerosis, heart attacks, and chronic fatigue syndrome, have been demonstrated. However, short-term oxidative stress, the unbalance between the formation and scavenging of the reactive oxygen species, may be important in the prevention of aging due to triggering of the process known as mitohormesis. On average, 65 - 70% of the population is excessively impacted by oxidative stress caused by FRs.



> MOS7 SENSITIV



## **DetectX**<sup>®</sup>

MULT PECIES

# **Glutathione (GSH) Colorimetric Detection Kits**

K006-H1 (4 Plate) K006-H1C-H/L (200 Cuvette)

FE	ATURES	
	Use	Measure Total GSH and GSSG to Determine Oxidative Stress
	Samples	Whole Blood, Serum, Plasma, Erythrocytes, Urine, Lysates, TCM
	Sensitivity	0.634 μM (Plate-based Format)
	Format	96-Well or Cuvette
	Species	Species Independent
•	Samples/Kit	K006-H1: 89 (Total and GSSG) in Duplicate K006-H1C-H/L: 43 (Total and GSSG) in Duplicate
	Stability	Liquid 4°C Stable Reagents
	Readout	Colorimetric, 405 nm

## SCIENTIFIC RELEVANCE

Glutathione (L-y-glutamyl-L-cysteinylglycine; GSH) is the highest concentration non-protein thiol in mammalian cells and is present in concentrations of 0.5 - 10 mM. GSH is a tripeptide that contains an unusual peptide linkage between the amine group of cysteine and the carboxyl group of the glutamate side-chain. It is an antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides. Glutathione reduces disulfide bonds formed within cytoplasmic proteins to cysteines by serving as an electron donor. In the process, glutathione is converted to its oxidized form, glutathione disulfide (GSSG). Glutathione is found mostly in its reduced form since the enzyme that reverts it from its oxidized form, glutathione reductase, is constitutive and inducible upon oxidative stress. The ratio of reduced glutathione to oxidized glutathione within cells is often used as a measure of cellular toxicity.





# **Glutathione (GSH) Fluorescent Detection Kits**

K006-F1 (1 Plate) | K006-F5 (5 Plate) K006-F1D (384-Well Plate)

## **FEATURES**

	Use	Measure GSH/GSSG to Determine Oxidative Stress	SPECIES
	Sample	Whole Blood, Serum, Plasma, Erythrocytes, Urine, Lysates, TCM	SENSITIVE
	Species	Species Independent	
	Sensitivity	45 nM Free GSH, 48 nM Total GSH	
•	Samples/Kit	39 or 231 in Duplicate (K006-F1/F5) 183 in Duplicate (K006-F1D)	
	Stability	Liquid 4°C Stable Reagents	
	Readout	Fluorescent, 510 nm em/370-410 nm ex	

## SCIENTIFIC RELEVANCE

Glutathione (L-y-glutamyl-L-cysteinylglycine; GSH) is the highest concentration non-protein thiol in mammalian cells and is present in concentrations of 0.5 - 10 mM. GSH is a tripeptide that contains an unusual peptide linkage between the amine group of cysteine and the carboxyl group of the glutamate side-chain. It is an antioxidant, preventing damage to important cellular components caused by reactive oxygen species, such as free radicals and peroxides. Glutathione reduces disulfide bonds formed within cytoplasmic proteins to cysteines by serving as an electron donor. In the process, glutathione is converted to its oxidized form, glutathione disulfide (GSSG). Glutathione is found mostly in its reduced form, since the enzyme that reverts it from its oxidized form, glutathione reductase, is constitutive and inducible upon oxidative stress. The ratio of reduced glutathione to oxidized glutathione within cells is often used as a measure of cellular toxicity.



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# **Glutathione Reductase Fluorescent Activity Kit**

K009-F1 (1 Plate)

## **FEATURES**

Use	Measure GR activity
Sample	RBCs, Serum, Plasma, Cells, Tissues
Convenient	20 minute End Point or Kinetic Assay
Sensitivity	9 µU/mL, World's Most Sensitive
Species	Species Independent
Samples/Kit	41 in Duplicate
Stability	Liquid 4°C Stable Reagents
Readout	Fluorescent, 510 nm em/370-410 nm ex



## SCIENTIFIC RELEVANCE

Glutathione reductase (GR) plays an indirect but essential role in the prevention of oxidative damage within the cell by helping to maintain appropriate levels of intracellular glutathione (GSH). GSH, in conjunction with the enzyme glutathione peroxidase (GP), is the acting reductant responsible for minimizing harmful hydrogen peroxide. The regeneration of GSH is catalyzed by GR. GR is a ubiquitous 100-120 kDa dimeric flavoprotein that catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione, using  $\beta$ -nicotinamide dinucleotide phosphate (NADPH) as the hydrogen donor. NADPH has been suggested to also act as an indirectly operating antioxidant, given its role in the recycling of GSSG to GSH and thus maintaining the antioxidative power of glutathione.





# **Glutathione S-Transferase Fluorescent Activity Kit**

K008-F1 (1 Plate)

## **FEATURES**

Use	Measure GST Activity
Sample	Serum, Plasma, Urine, Cell Lysates
Samples/Kit	40 in Duplicate
Convenient	30 Minute End Point or Kinetic Assay
Sensitivity	< 100 $\mu$ U of GST Activity
Stability	Liquid 4°C Stable Reagents
Readout	Fluorescent, 460 nm em/370-410 nm



## SCIENTIFIC RELEVANCE

The Glutathione S-Transferase (GST) family of isozymes function to detoxify and neutralize a wide variety of electrophilic molecules by mediating their conjugation with reduced glutathione. Human GSTs are encoded by five gene families, expressing in almost all tissues as four cytosolic and one microsomal forms. Given its pivotal role in ameliorating oxidative stress/damage, GST activity has been repeatedly investigated as a biomarker for arthritis, asthma, COPD, and multiple forms of cancer, as well as an environmental marker. Examination of GST isoforms and activity in human cancers, tumors and tumor cell lines has revealed the predominance of the acidic pi class. Furthermore, this activity is thought to substantially contribute to the innate or acquired resistance of specific neoplasms to anticancer therapy.

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# **Hemoglobin Colorimetric Detection Kits**

Regular: K013-H1 (2 Plate) High Sensitivity: K013-HX1 (2 Plate) | K013-HX5 (10 Plate)

## FEATURES

	Sample Type	K013-H: Whole Blood, RBCs K013-HX: Serum, Plasma
	Time to Answer	30 Minutes
•	Range	K013-H: 16-0.25 g/dL K013-HX: 20-0.313 µg/mL
•	Sensitivity	K013-H: 0.021 g/dL, 0.21 mg/mL K013-HX1: 0.053 ug/mL
	Samples/Kit	88 in Duplicate
	Stable	Liquid 4°C Stable Reagents
	Readout	K013-H: 560-580 nm K013-HX: 450 nm



#### SCIENTIFIC RELEVANCE

Hemoglobin (Hgb) is an erythrocyte protein complex comprised of two sets of identical pairs of subunits, each of which bind an iron-porphyrin group commonly called heme. Heme binds and releases oxygen or carbon dioxide in response to slight changes in local gas tension. Hemoglobin values are associated with a variety of conditions ranging from anemias (low Hgb), erythrocytosis (high Hgb), thalassemias (aberrant chain synthesis), and sickling disorders (abnormal complex shape).



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# Hydrogen Peroxide Colorimetric & Fluorescent Detection Kits

Colorimetric: K034-H1 (2 Plate) Fluorescent: K034-F1 (2 Plate) **FEATURES** 

	Use	Measure $H_2O_2$ in Any Sample
	Sample	Urine, Buffer, TCM
	Rapid	15 Minutes
•	Sensitive	Colorimetric: 91.3 pmol (310 ng) Fluorescent: < 2 pmole (65 pg)
•	Samples/Kit	Colorimetric: 89 in Duplicate Fluorescent: 88 in Duplicate
	Readout	Colorimetric: 560 nm Fluorescent: 590 nm em/520 nm ex

#### SCIENTIFIC RELEVANCE

In biological systems, incomplete reduction of O<sub>2</sub> during respiration produces superoxide anion  $(O_2^{-})$ , which is spontaneously or enzymatically dismutated by superoxide dismutase to H<sub>2</sub>O<sub>2</sub>. Many cells produce low levels of O<sub>2</sub><sup>--</sup> and H<sub>2</sub>O<sub>2</sub> in response to a variety of extracellular stimuli, such as cytokines (TGF- $\beta$ 1, TNF- $\alpha$ , and various interleukins), peptide growth factors (PDGF, EGF, VEGF, bFGF, and insulin), the agonists of heterotrimeric G protein-coupled receptors (GPCR) such as angiotensin II, thrombin, lysophosphatidic acid, sphingosine 1-phosphate, histamine, and bradykinin, and by shear stress. The addition of exogenous H2O2,  $\frac{1}{2}$ or the intracellular production in response to receptor stimulation, affects the function of various proteins including protein kinases, protein phosphatases, transcription factors, phospholipases, ion channels, and G proteins. In 1894, Fenton described the oxidation of tartaric acid by  $Fe^{2+}$  and  $H_2O_2$ .  $H_2O_3$ and O<sub>2</sub> may participate in the production of singlet oxygen



and peroxynitrite and the generation of these species may be concurrent with reactions involving iron, which under some circumstances might be important contributors to  $H_2O_2$  toxicity.



# Myeloperoxidase (MPO) Human ELISA Kit

K060-H1 (1 Plate)

#### **FEATURES**

► Use Measure Human MPO in a Variety of Matrices

Sample Serum, Platelet-Poor Heparin Plasma, Saliva, Urine, TCM

- Time to Answer 2.5 Hours
- Sensitivity
  0.068 ng/mL, 68 pg/mL
- Species
  Human
- Samples/Kit 40 in Duplicate
- Readout
  Colorimetric, 450 nm

#### SCIENTIFIC RELEVANCE

Myeloperoxidase (MPO) is a tetrameric heme-containing protein abundantly produced in neutrophil granulocytes where it plays an important anti-microbial role. During degranulation MPO is released into the extracellular space. There, as part of the neutrophils "respiratory burst", it produces hypochlorous acid from hydrogen peroxide and Cl<sup>-</sup>. MPO also uses hydrogen peroxide to oxidize tyrosine to the tyrosyl radical. Both hypochlorous acid and tyrosyl are cytotoxic and when present can kill bacteria and other pathogens. Hereditary deficiency of myeloperoxidase predisposes individuals to immune deficiency. Studies have shown an association between elevated MPO levels and coronary artery disease, and in 2003 it was suggested that MPO may serve as a sensitive



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predictor of myocardial infarction in patients complaining of chest pain. Since that time the clinical utility of MPO testing in cardiac patients has been solidly established in the literature with well over 100 papers published. In 2010 this clinical application was further refined by additional studies which determined that measuring both MPO and C-reactive protein (CRP) provided more accurate prediction of mortality risk than measuring just CRP alone.



# **Nitric Oxide Colorimetric Detection Kit**

#### K023-H1 (2 Plate)

## FEATURES

Use	Measure Nitrite & Nitrate
Sample	Water, Serum, Plasma, Urine, Saliva, Lysates, Buffers, TCM
Accurate	Calibrated to NIST Standard Reference Material #3185
Sensitivity	Highest Optical Density of Any Kit
Time to Answer	5 Minute Nitrite – 25 Minute Total NO
Samples/Kit	88 in Duplicate
Stability	Non-Toxic, Liquid 4°C Stable Reagents
Readout	Colorimetric, 550-570 nm



## SCIENTIFIC RELEVANCE

Nitric oxide (NO) is a diffusible, transient, reactive molecule that has physiological effects in the pM-µM range. Acting through guanylate cyclase activation, NO is an important regulator of the and immunological cardiovascular, nervous, systems. NO is bio-available by two routes. It can be endogenously generated by constitutive or induced NOS enzymes, or it can be ingested as nitrates or nitrites for conversion into NO. The reactive nature of nitric oxide allows it to act as a cytotoxic factor when released during an immune response by macrophages. The reactivity also allows NO to be easily converted to a toxic radical that can produce nitrosylation damage to cells and DNA. Nitrosylation can be a regulated post-translational modification in cell signaling. The dynamics of the regulatory/



damage facets of NO are major forces in mitochondrial signaling and dysfunction. NO is linked not only to coronary heart disease, endothelial dysfunctions, erectile dysfunction, and neurological disorders, but also diabetes, chronic periodontitis, autism and cancer.



# Superoxide Dismutase (SOD) Activity Kit

#### K028-H1 (2 Plate)

## FEATURES

- Use
  Oxidative Stress Determination
- Sample Serum, Plasma, Cells, Tissue Buffers, Erythrocytes
- Species Human and Other Mammalian Species
- Samples/Kit 88 in Duplicate
- Time to Answer
  20 Minutes
- Readout
  Colorimetric, 450 nm



## SCIENTIFIC RELEVANCE

Short-lived and highly reactive oxygen species (ROS) such as O<sub>2</sub><sup>-</sup> (superoxide), <sup>-</sup>OH (hydroxyl radical), and H<sub>2</sub>O<sub>2</sub> (hydrogen peroxide) are continuously generated in vivo. The cellular levels of ROS are controlled by antioxidant enzymes and small molecule antioxidants. The major antioxidant enzymes, superoxide including dismutases (SODs), copperzinc superoxide dismutase (Cu/ZnSOD), manganese superoxide dismutase (MnSOD), and extracellular superoxide dismutase (EC-SOD). All play a critical roles in scavenging  $O_{a}^{-1}$ ·. Decreased SOD activity results in elevated level of superoxide which in turn leads to decreased NO and increased peroxynitrite concentrations. The major intracellular SOD is a 32-kDa copper and zinc containing homodimer (Cu/Zn SOD). The mitochondrial



SOD (MnSOD) is a manganese-containing 93-kDa homotetramer that is synthesized in the cytoplasm and translocated to the inner matrix of mitochondria. EC-SOD is the primary extracellular SOD enzyme and is highly expressed in many organs. Increased SOD activity levels are seen in Downs Syndrome, while decreased activity is seen in diabetes, Alzheimer's disease, rheumatoid arthritis, Parkinson's disease, uremic anemia, atherosclerosis, some cancers, and thyroid dysfunction.



# **TBAR MDA Universal Colorimetric Detection Kit**

K077-H1 (2 Plate)

## FEATURES

Use	Assess lipid peroxidation without boiling water bath treatment
Sample	Serum, Plasma, Tissue, Cell and Food Extracts, Urine, Buffers
Sensitivity	0.36 µMf
Time to Answer	1 Hour
Format	Shaking at 37°C
Samples/Kit	88 in Duplicate
Stability	Liquid 4°C Stable Reagents
Readout	Colorimetric, 535 nm



## SCIENTIFIC RELEVANCE

Malondialdehyde (MDA) is a 3-carbon dialdehyde formed by lipid peroxidation of polyunsaturated fatty acids by reactive oxygen species and other oxidative machanisms. Lipid peroxidation is a wellestablished mechanism of cellular injury in plants and animals and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxidation products derived from polyunsaturated fatty acids decompose to form a diverse mixture of compounds, including MDA. The combined total of MDA plus other reactive substances are termed TBARS for Thiobarbituric Acid Reactive Substances. Modifications of the TBARS assay have been used to evaluate several types of samples, including mammalian tissues, serum, plasma and urine along with food samples. There is some ambiguity surrounding the use of TBARS in different sample types under different oxidative stress because of the reactivity of acidified TBA toward reactive aldehydes, however the assay is used extensively to determine lipid peroxidation. In general lipids with greater unsaturation will yield higher TBARS values.



# **Thiol Fluorescent Detection Kits**

#### K005-F1 (1 Plate)

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## **FEATURES**

- Use Measure Thiol Content of Proteins and Peptides
- Adaptable
  Measure SH easily in 8M GuHCl Buffers
- Sensitivity
  4.62 nM
- Time to Answer 30 Minutes
- Species
  Species Independent
- Samples/Kit 39 in Duplicate
- Stability Liquid
  4°C Stable Reagents
- ► Readout Fluorescent, 510 nm em/370-410 nm ex

## SCIENTIFIC RELEVANCE

Free thiols in biological systems have important roles. Oxidatively-modified thiol groups of cysteine residues are known to modulate the activity of a growing number of proteins. As such, it is important to be able to accurately determine the extent of modification of specific amino acids, such as cysteine residues. This is especially difficult in a complex protein sample, especially in the presence of chaotropic agents such as guanidine hydrochloride. Typical methods using Ellman's reagent do not have sufficient sensitivity to allow economical detection of free SH groups.





www.ArborAssays.com | info@ArborAssays.com

Ph: +1-734-677-1774

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