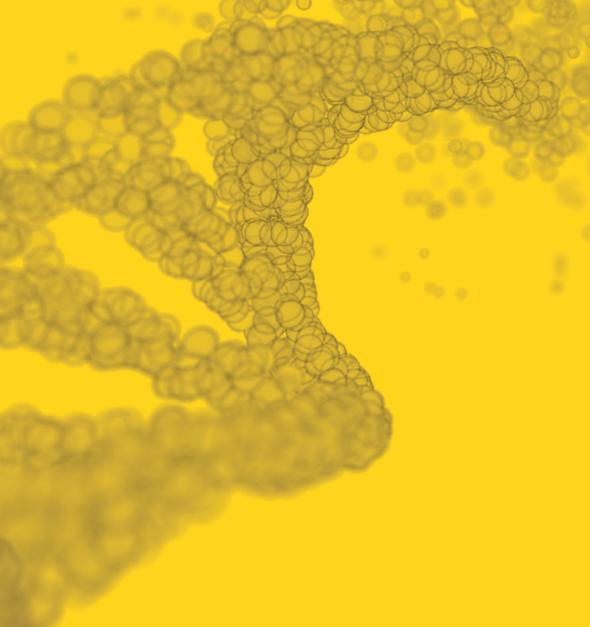
GUIDE

# **Oxidative Stress**

**Markers & Detection Tools** 





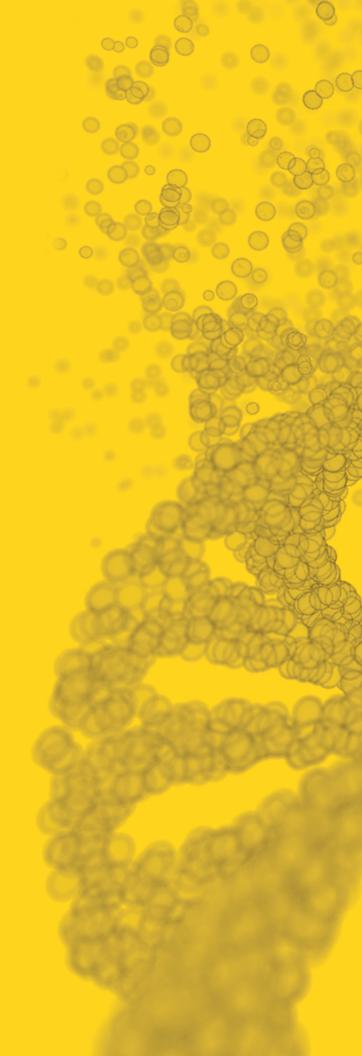
Oxidative stress occurs when there is an imbalance between the production of chemically reactive species such as reactive oxygen, nitrogen, and sulfur species (ROS, RNS, RSS) and antioxidant defense mechanisms. Uncontrolled oxidation can disrupt redox signaling and cause injury to cellular components like lipids, proteins, and nucleic acids, resulting in irreparable damage and eventual cell death.

Oxidative stress can be evaluated directly by measuring reactive species or indirectly by the associated damage to lipids, proteins, and nucleic acids. Although direct measurement of these reactive species is desirable, indirect methods are often more reliable due to the relative stability of damage markers on biomolecules compared to the transient nature of reactive species. Multiple biomarkers of oxidative damage have been identified for different macromolecules (e.g., proteins, lipids, and DNA/RNA).

Use this guide to find the right marker of oxidative stress and detection tool for your application.

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## **Method Considerations**

Because of their high reactivity, ROS, RNS, and RSS are transient: they have short half-lives and are typically present at low concentrations, necessitating the use of sensitive detection methods. There are many approaches for detecting reactive species and their damage markers. Key factors to consider when selecting a method include:

- Reproducibility
- · Type of reactive species
- Reaction chemistry
- Specificity

- · Qualitative versus quantitative approaches
- Interfering compounds
- Technical expertise
- · Equipment available



### **Ready-made Assay Kits**

Ready-made assay kits are a convenient and simple tool for the detection of reactive species or markers of oxidative stress. Assay kits are validated and pre-optimized, saving you valuable time and resources.

#### **Benefits**

- ✓ Reproducible
- ✓ Easy to use
- ✓ Includes protocols & reagents
- ✓ High sample throughput
- Choice of application (e.g., ELISA, flow cytometry (FC), fluorescent microscopy)



#### Fluorescent Probes

Fluorescent probes are valuable tools but tend to be non-specific for any given species. A secondary method should be used to confirm results using fluorescent probes.

#### Benefits

- ✓ Easy to use
- ✓ Sensitive
- ✓ Wide range of fluorophores available
- Scavengers available to improve specificity (e.g., catalase for H<sub>2</sub>O<sub>2</sub>)
- Choice of application (e.g., FC, fluorescent microscopy, microplate reader)



## **Mass Spectrometry**

Some markers of oxidative stress can be detected by mass spectrometry (LC-MS or GC-MS). There have been significant advances in MS workflows, yet it remains a highly technical, time-intensive approach that requires extensive skill.

#### Benefits

- ✓ Broad coverage
- ✓ Specific

- ✓ Sensitive
- ✓ Flexible workflows

## Oxidative Stress LC-MS Mixture

Item No. 18701

· Contains a mixture of lipids and nucleic acids produced during oxidative stress





### **Antibody-based Techniques**

Other markers of oxidative stress can be detected with antibody-based techniques like Western blot (WB), immunocytochemistry (ICC), or immunohistochemistry (IHC). WB is useful for determining abundance of a protein biomarker, and ICC and IHC can elucidate marker distribution and localization but are generally considered qualitative measures.

#### **Benefits**

- ✓ Target protein of interest
- ✓ Determine cellular localization
- ✓ Potential use with archived tissues



## **Spin Traps**

Spin traps capture free radicals, producing a spin adduct that can be detected by electron spin resonance (ESR). The spin adduct has a distinct spectrum that allows for the identification of the trapped radical. While often regarded as the gold standard for the direct detection of reactive species, not all researchers have access to an ESR instrument.

#### **Benefits**

- ✓ Sensitive
- ✓ Specific

- ✓ High stability of spin adduct
- ✓ Potential *in vivo* applications

#### **Spin Traps**

Item No.	Product Name
14958	ВМРО
10009660	СҮРМРО
10006435	DEPMPO
10006436	DMPO
20618	EMPO
14982	PTIO
81540	Carboxy-PTIO (potassium salt)
14877	TEMPONE

View additional spin traps online at www.caymanchem.com



# Stressed about Picking an Oxidative Damage Assay?

Get dependable results with reliable assays. Explore the trusted methods used in Cayman's oxidative damage assays.

www.caymanchem.com/oxidativedamage

## Bioanalysis & Assay Development

Cayman's Bioanalysis & Assay Development Services leverage the expertise of our scientists to aid your research and/or drug discovery efforts.





www.caymanchem.com/bioanalysis

## ROS

ROS are produced from molecular oxygen during normal cellular processes or in response to harmful exogenous factors. ROS have central roles in redox regulation and cell signaling, but excessive ROS production triggers oxidative stress. Examples of ROS include superoxide  $(O_2^{\bullet})$ , hydrogen peroxide  $(H_2O_2)$ , and the hydroxyl radical  $(OH^{\bullet})$ .

#### **ENDOGENOUS FACTORS**



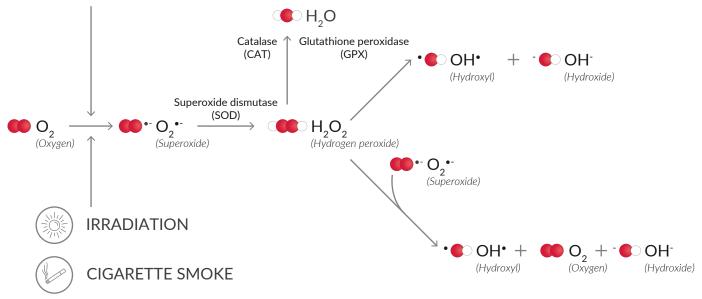
#### **CELLULAR METABOLISM**

(mitochondria, xanthine oxidase, peroxisomes)



#### INFLAMMATION

(neutrophils, monocytes/macrophages)





DRUGS



**ENVIRONMENTAL TOXINS** 



#### **EXOGENOUS FACTORS**





 $O_2^{\bullet \bullet}$  is the precursor for many ROS. It is rapidly converted to  $H_2O_2$  by superoxide dismutase (SOD).

## $H_2O_2$



 ${
m H_2O_2}$  is relatively stable compared to other ROS, making it more readily detectable. Although it is less reactive than other ROS,  ${
m H_2O_2}$  can easily diffuse across biological membranes to injure nearby cells.

#### OH.



OH• is the strongest and most injurious ROS. It is highly reactive and can damage lipids, proteins, and DNA.

Find small molecule inhibitors for ROS at www.caymanchem.com



Free radical generators & ROS-producing probes available at www.caymanchem.com

#### **ROS Assay Kits**

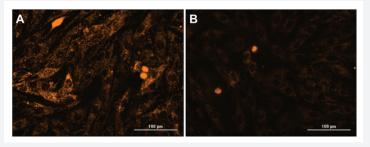
Item No.	Product Name	Format	Sample Types	Key Features
600050	Hydrogen Peroxide Assay Kit	Plate-based fluorometric (Ex/Em = 530-560/590 nm) or colorimetric (570 nm) assay	Cell samples	Includes catalase as specificity control
701600	Mitochondrial ROS Detection Assay Kit	Plate-based fluorometric assay, FC (Ex/Em = 480-515/560-600 nm)	Adherent or suspension cells	Includes positive and negative controls
601520	ROS Detection Cell-Based Assay Kit (DCFDA)	Plate-based fluorometric assay, FC (Ex/Em = 480-500/510-550 nm)	Adherent or suspension cells	Includes positive and negative controls
601290	ROS Detection Cell-Based Assay Kit (DHE)	Plate-based fluorometric assay, FC (Ex/Em = 480-520/570-600 nm)	Adherent or suspension cells	Includes positive and negative controls

## Mitochondrial ROS Detection Assay Kit

Item No. 701600

#### Features:

- Measure mitochondrial ROS directly in living cells
- Includes antimycin A as positive control
- Flexible: Compatible with FC, fluorescence, and plate-based fluorometric formats (Ex/Em = 480-515/560-600 nm)



H9c2 cells were stained with 0.62  $\mu$ M Mitochondrial ROS Detection Reagent following the Adherent Cells Protocol treated with 3  $\mu$ M antimycin A (A) or vehicle (B). Images were captured one hour after treatment with antimycin A using Biotek's Cytation<sup>TM</sup> 5 Multi-Mode Reader.

## MitoCheck® Assays

Measure the activity of complexes I-V of the electron transport chain with Cayman's MitoCheck® Assays.



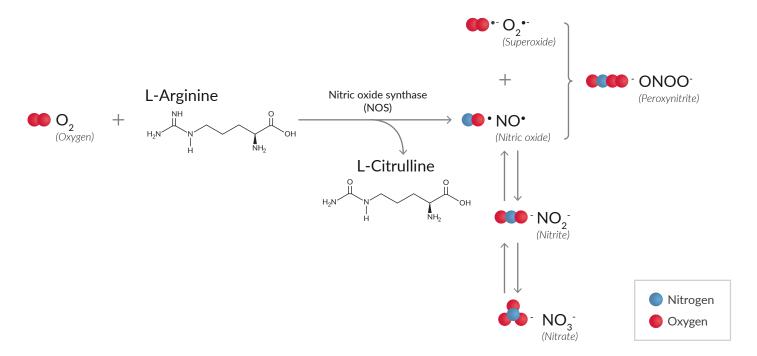
#### **ROS Probes**

Item No.	Product Name	Detects	Excitation (nm)	Emission (nm)	Key Features
10010469	10-Acetyl-3,7- dihydroxyphenoxazine (ADHP)	H <sub>2</sub> O <sub>2</sub>	<b>520-550</b>	■ - ■ 585-595	A sensitive, stable substrate for HRP that detects $\rm H_2O_2$
20656	2',7'-Dichlorofluorescein diacetate	Non-specific ROS	<b>4</b> 92	<b>515</b>	Rapidly de-esterified and oxidized in cells to form the fluorescent product 2'7'-dichlorofluorescein
10157	APF	OCI <sup>-</sup> , OH*, ONOO <sup>-</sup> , <sup>1</sup> O <sub>2</sub>	<b>4</b> 90	<b>5</b> 15	Low intrinsic fluorescence
12013	Dihydroethidium	O <sub>2</sub> • - & other oxidants	<b>4</b> 90	<b>5</b> 90	Also detectable by HPLC
27307	Homovanillic Acid	H <sub>2</sub> O <sub>2</sub>	<b>3</b> 12	<b>420</b>	Forms a fluorescent dimer upon oxidation
14872	Lucigenin	H <sub>2</sub> O <sub>2</sub> , O <sub>2</sub> •-	Chemiluminescent		Fluorescence is also quenched by chloride
16803	Luminol	Non-specific ROS	Chemiluminescent		Chemiluminescence reaction can be enhanced with <i>p</i> -substituted phenols
25169	MitoROS™ 580	O <sub>2</sub> •-	<b>5</b> 10	<b>580</b>	Targets mitochondria
10005983	Pentafluorobenzenesulfonyl fluorescein	H <sub>2</sub> O <sub>2</sub>	<b>485</b>	<b>5</b> 30	Fluoresces upon perhydrolysis of the sulfonyl linkage and is selective for ${\rm H_2O_2}$

 $\label{thm:composition} \textit{View additional ROS probes online at www.caymanchem.com}$ 

## **RNS**

RNS are also produced during oxidative stress. Nitric oxide synthase (NOS) uses L-arginine and  $O_2$  to produce nitric oxide (NO $^{\bullet}$ ), which can be oxidized to other RNS. The reaction between NO $^{\bullet}$  and  $O_2^{\bullet-}$  forms peroxynitrite (ONOO $^{-}$ ), a strong oxidant and nitrating agent.



#### NO.



NO• is a signaling molecule and RNS that can rapidly diffuse across cell membranes. Since it is shortlived and physiological concentrations are typically low, NOS activity assays are often used as an indirect measurement of cellular NO• production.

## NO<sub>2</sub>-/NO<sub>3</sub>-



NO<sub>2</sub>-/NO<sub>3</sub> are degradation products of NO• that can be converted back to NO•. These products are more stable than NO• and are used as an indirect measurement of NO• production.

#### ONOO.



ONOO is a potent oxidant and antimicrobial and cytotoxic agent. It can react with tyrosine residues to form nitrotyrosine (pg. 10), a marker of nitrative stress.

## **Nitric Oxide Donors**

#### **DETA NONOate**

Item No. 82120

A NO donor that spontaneously dissociates in a pH-dependent, first-order process to liberate two moles of NO per mole of parent compound.

-O N N NH<sub>2</sub>

See all nitric oxide donors at www.caymanchem.com

Discover Cayman's small molecule inhibitors for RNS at www.caymanchem.com

#### **RNS Assay Kits**

Item No.	Product Name	Format	Sample Types	Key Features
780001	Nitrate/Nitrite Colorimetric Assay Kit*	Plate-based colorimetric assay (540-550 nm)	Plasma, serum, urine, cell supernatant, & tissue homogenates	Avoids NADPH interference by using a catalytic system to recycle spent NADP+ back to NADPH
760871	Nitrate/Nitrite Colorimetric Assay Kit (LDH method)	Plate-based colorimetric assay (530-550 nm)	Plasma, serum, urine, & tissue homogenates	Includes LDH to remove interfering NADPH
781001	NOS Activity Assay Kit	Scintillation	Cell lysates & purified preparations	Monitors the conversion of arginine to citrulline by NOS

<sup>\*</sup> Item No. 780051 is available as a fluorometric alternative

#### **RNS Probes**

Item No.	Product Name	Detects	Excitation (nm)	Emission (nm)	Key Features
85155	2,7-Dichlorodihydrofluorescein diacetate	ONOO-	<b>5</b> 02	<b>5</b> 23	Does not appear to be oxidized by NO $^{\bullet}$ , H $_2$ O $_2$ , or O $_2$ $^{\bullet}$ alone
14051	Coumarin Boronic Acid	ONOO-, OCI-, H <sub>2</sub> O <sub>2</sub>	■ 332	<b>47</b> 0	Reacts with ONOO at a faster rate than $\rm H_2O_2$ or OCl $$
10818	Coumarin Boronic Acid pinacolate ester	ONOO-, OCI-, H <sub>2</sub> O <sub>2</sub>	<b>3</b> 32	<b>470</b>	More soluble form of Item No. 14051
85160	DAF-2	NO•	<b>485</b>	<b>5</b> 38	NO• detection limit: ~5 nM
85165	DAF-2 diacetate	NO•	■ 485	<b>538</b>	Improved cell permeability over Item No. 85160; NO• detection limit: 2-5 nM (at neutral pH)
18767	DAF-FM diacetate	NO•	<b>4</b> 95	<b>515</b>	NO• detection limit: ~3 nM
85070	DAN-1 EE (hydrochloride)	NO.	<b>- 360-380</b>	<b>-</b> 420-450	A cell-permeable form of DAN
85100	Dihydrorhodamine 123	ONOO-	<b>5</b> 00	<b>5</b> 36	Does not appear to be oxidized by NO*, $\rm H_2O_2$ , or $\rm O_2^{\bullet^-}$ alone

View additional RNS probes online at www.caymanchem.com

#### **RNS Antibodies**

Item No.	Product Name	Key Features
160862	iNOS Polyclonal Antibody	Host: Rabbit · Reactivity: Mouse · Applications: IHC, IP, WB
160870	nNOS Polyclonal Antibody	Host: Rabbit · Reactivity: Human, rat · Applications: ICC, IHC, WB
160880	eNOS Polyclonal Antiserum	Host: Rabbit · Reactivity: Human, bovine · Applications: WB

## **Cellular Metabolism Assays**

Explore therapeutic avenues in various disease models using high-content imaging, enzymatic screening, and the Agilent Seahorse™ XF Pro.

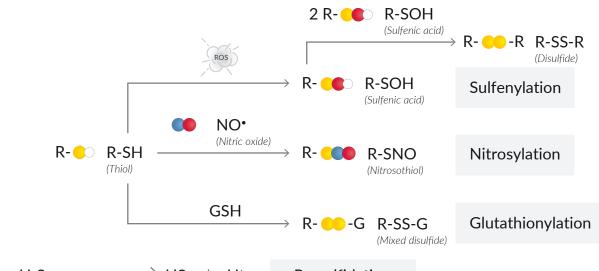
- Mitochondrial biogenesis & function
- ROS generation & oxidative stress
- Immunometabolism studies
- · Custom cellular models of disease



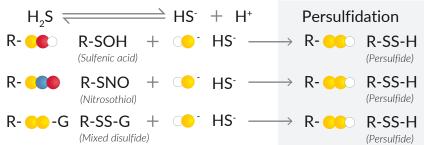


## RSS

RSS are redox-active sulfur-based compounds. They may be inorganic RSS, like hydrogen sulfide ( $H_2S$ ), or organic RSS, which may be formed by the oxidation of cysteine thiol groups (R-SH) or *via* reactions between  $H_2S$  and other RSS. Oxidative modifications to proteins like disulfide formation, sulfenylation, nitrosylation, glutathionylation, or persulfidation can lead to altered protein structure and/or function. Simplified select RSS reactions are shown below.



R- ( )





## R-SOH R-

R-SOH is highly reactive. It is formed by the reaction of R-SH and various ROS, resulting in a protein modification known as sulfenylation. R-SOH is rapidly converted to other RSS, like sulfinic or sulfonic acids and protein disulfides (R-SS-R).

## R-SNO

R-SNO is formed by the reaction of R-SH and NO\*, forming nitrosothiol, a protein modification known as nitrosylation. The biotin-switch technique is commonly used method to detect nitrosylated proteins.

## R-SS-G

R-SS-G is formed by the reaction of R-SH with glutathione (GSH), a process that forms glutathionylated proteins.

R- -G

## H<sub>2</sub>S/HS<sup>-</sup> · · · · / · · ·

H<sub>2</sub>S exists predominantly in the anionic form (HS<sup>-</sup>) at physiological pH.
HS<sup>-</sup> reacts with other
RSS to form persulfides
(R-SS-H), a protein modification known as persulfidation.

#### MitoA

Item No. 22702

A mitochondria-targeted mass spectrometry probe that can be used to assess relative changes in mitochondrial matrix H<sub>2</sub>S concentration.

#### **RSS Probes**

Item No.	Product Name	Detects	Excitation (nm)	Emission (nm)	Key Features
35589	PSP	H <sub>2</sub> S <sub>n</sub>			A two-photon fluorescent probe
11179	WSP-1	H <sub>2</sub> S	<b>465</b>	<b>5</b> 15	A turn-on fluorescent probe
16929	WSP-5	H <sub>2</sub> S	<b>5</b> 02	<b>5</b> 25	Faster turn-on rate & improved sensitivity for H <sub>2</sub> S over Item No. 11179

#### **Thiol-reactive Probes**

Monitor thiol depletion in response to oxidative stress with these thiol-reactive probes.

Item No.	Product Name	Excitation (nm)	Emission (nm)
34564	7-Fluoro-2,1,3-benzoxadiazole-4-sulfonate (ammonium salt)	<b>380</b>	<b>515</b>
17097	Monobromobimane	<b>3</b> 98	<b>4</b> 90
13083	ThioFluor 623	<b>5</b> 63	<b>623</b>
13235	ThioGlo1	<b>384</b>	<b>513</b>

#### S-Nitrosothiol Reagents

These reagents can be used in the biotin-switch technique to tag S-nitrosylated proteins.

Item No.	Product Name
16459	Biotin-HPDP
14656	L-Ascorbic Acid
9002267	MTSEA
17237	MTSEA-biotin
16529	MTSES
17215	SNOB 1 Reagent

# Measure Free Thiol Content

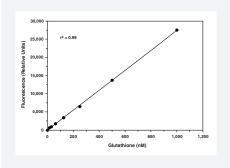
## Thiol Detection Assay Kit

Item No. 700340

A simple, sensitive, and reproducible assay for free thiol content in a variety of sample types.

Format: Plate-based fluorometric assay

(Ex/Em = 380-390/510-520 nm)



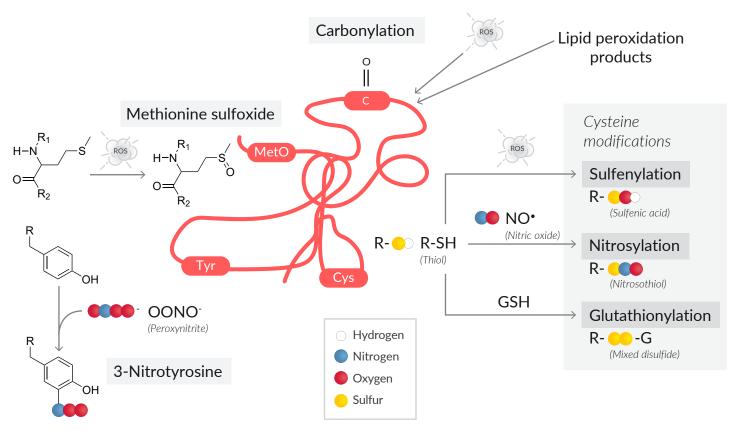
### **Sulfenic Acid Clickable Tags**

Isolate and identify sulfenic acid-modified proteins with these clickable tags available from Cayman.

Item No.	Product Name	Key Features
13038	Alkynyl-biotin	Can be used to capture sulfenic acid-modified proteins tagged with DAz-2
21287	BCN-E-BCN	Selective over free thiol, sulfinic, or sulfonic forms of proteins
13173	DAz-1	Cell-permeable & can be conjugated to biotin or various fluorophores for detection
13382	DAz-2	Cell-permeable & more sensitive than DAz-1
11220	DYn-2	Can be used with intact cells
13581	Phosphine-biotin	Has been used in conjunction with DAz-1 & DAz-2

## **Protein Oxidative Modifications**

ROS, RNS, and RSS react with proteins, resulting in modifications to various amino acid residues that can alter the structure and/or function of cellular proteins. These reactive species can either directly oxidize amino acids, especially those containing thiol groups like methionine or cysteine, or introduce carbonyl groups in the side chains of certain amino acids.



## Carbonylation

Protein carbonylation is the most common and general marker of protein oxidation. It is induced by reactive species and lipid peroxidation products. Protein carbonylation introduces a reactive carbonyl group (e.g., aldehydes, ketones) into a protein.

## Methionine sulfoxide

ROS damage at methionine residues produces methionine sulfoxide (MetO). MetO reductases can reverse this modification.

## 3-Nitrotyrosine

3-Nitrotyrosine is a marker of ONOO. Tyrosine, an aromatic amino acid containing a hydroxyl group, is susceptible to nitration.

## Proteomics & Post-Transitional Modification Analyses

With support from our facilities and through our partnership with MS Bioworks, we offer full-service targeted and untargeted conventional and mass spectrometry-based proteomics services ranging from immunoprecipitation to data analysis.



www.caymanchem.com/bioanalysis

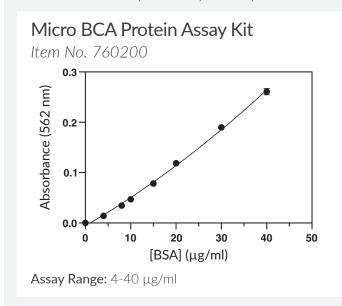
#### **Protein Modification Kits**

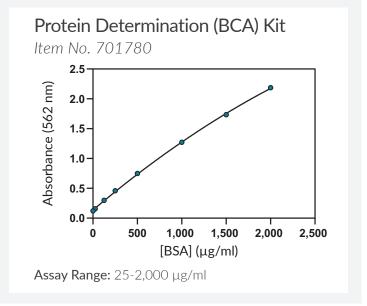
Item No.	Product Name	Format	Samples Types	Key Features
600160	Methionine Sulfoxide Immunoblotting Kit	WB	Cell or tissue lysates, semi-pure or purified proteins	Uses an antibody with minimal cross- reactivity to methionine sulfone
10005020	Protein Carbonyl Colorimetric Assay Kit*	Plate-based colorimetric assay (360-385 nm)	Plasma, serum, tissue homogenates, & cell lysates	Utilizes DNPH for reaction chemistry
10010721	S-Glutathionylated Protein Detection Kit	FC or fluorescent microscopy (Ex/Em = 488/518-535 nm)	Cells	Uses a modified biotin-switch method
10006518	S-Nitrosylated Protein Detection Kit (Biotin Switch)	Plate-based colorimetric (substrate-dependent) or fluorometric (Ex/Em = 490/520 nm) assay, or fluorescent microscopy	Cells or tissues	Uses a modified biotin-switch method
600320	Sulfenylated Protein Cell-Based Detection Kit	Plate-based fluorometric assay, fluorescent microscopy, or FC (Ex/Em = 485/535 nm)	Cells	Can be used with viable cells

<sup>\*</sup> Item No. 701530 is available as a fluorometric alternative

## **Complementary Kits**

Determine the total protein concentration in your samples with these plate-based colorimetric assays (562 nm). Knowing the concentration of protein in your samples allows for normalization of results between samples.



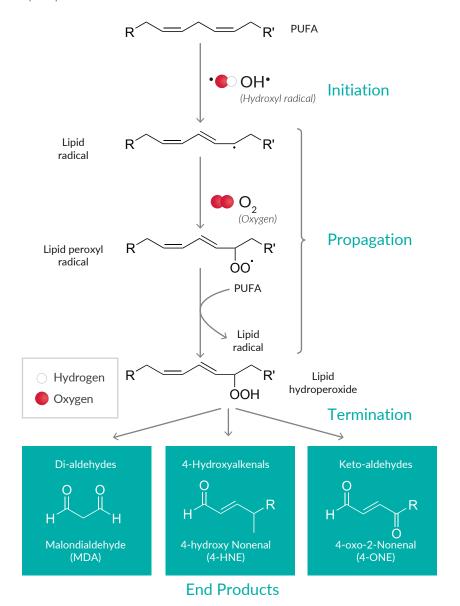


### **Nitrotyrosine Antibodies**

Item No.	Product Name	Key Features
389549	Nitrotyrosine Affinity Sorbent	For immunoprecipitation of nitrotyrosine-containing proteins
601220	Nitrotyrosine IP Kit	For the capture & pulldown of nitrated proteins
10006778	Nitrotyrosine (Peptide) Polyclonal Antibody	Host: Rabbit · Reactivity: Species independent · Applications: WB
189542	Nitrotyrosine Monoclonal Antibody	Host: Mouse · Reactivity: Species independent · Applications: ELISA, WB
10006966	Nitrotyrosine Monoclonal Antibody - Biotinylated	Host: Mouse · Reactivity: Species independent · Applications: ELISA, WB
10189540	Nitrotyrosine Polyclonal Antibody	Host: Rabbit · Reactivity: Species independent · Applications: WB

# **Lipid Peroxidation**

Lipid peroxidation is most often initiated by ROS like OH\*, which target polyunsaturated fatty acids (PUFAs). This initiates a chain reaction that results in the formation of highly reactive and unstable lipid hydroperoxides (LPOs). LPOs are rapidly degraded into various products (e.g., alkanes, ketones, aldehydes) like malondialdehyde (MDA) and 4-hydroxy nonenal (4-HNE) that can be assayed as markers of lipid peroxidation.



#### **LPOs**

LPOs can be measured either directly or assessed indirectly by the various decomposition products.

#### **MDA**

MDA assays use a thiobarbituric acid reaction and are thus referred to as Thiobarbituric Acid Reactive Substances (TBARS) assays. Thiobarbituric acid reacts with various aldehydes produced during lipid peroxidation in addition to MDA.

#### 4-HNE

4-HNE protein adducts are typically more stable than MDA protein adducts. 1,4-Dihydroxynonane mercapturic acid (DHN-MA), the major urinary metabolite of 4-HNE, is an additional biomarker that may be assayed.

## **Explore Ferroptosis:**

# An Iron-Dependent Form of Cell Death Driven by Lipid Peroxidation

Learn more about ferroptosis and download or request a physical copy of our ferroptosis wall poster.

COPY

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www.caymanchem.com/ferroptosis

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#### **Lipid Peroxidation Assay Kits**

Item No.	Product Name	Format	Sample Types	Assay Range	Key Features
705002	Lipid Hydroperoxide (LPO) Assay Kit*	Plate-based colorimetric assay (500 nm)	Tissues, cells, plant materials, food, & biological fluids	0.25-5 nmol hydroperoxide/tube	Extraction protocol removes most interfering substances
501140	DHN-MA-EIA Kit	Plate-based colorimetric assay (405 or 414 nm)	Urine	7.8-1,000 pg/ml	Can be measured without extraction
10009055	TBARS Assay Kit	Plate-based colorimetric (530-540 nm) or fluorometric (Ex/Em = 530/550 nm) assay	Plasma, serum, urine, tissue homogenates, & cell lysates	Colorimetric: 0.625-50 μM Fluorometric: 0.0625-5 μM	Flexible platform
700870	TBARS (TCA Method) Assay Kit	Plate-based colorimetric (530-540 nm) or fluorometric (Ex/Em = 530/550 nm) assay	Plasma, serum, urine, tissue homogenates, & cell lysates	Colorimetric: 0.625-50 μM Fluorometric: 0.0625-5 μM	Includes sample acid precipitation protocol to avoid confounding soluble TBARS

<sup>\*</sup>Item No. 705003 is designed for use with a 96-well reusable glass plate

#### 8-Isoprostane Assay Kits

Item No.	Product Name	Format	Sample Types	Assay Range	Key Features
516351	8-Isoprostane ELISA Kit	Plate-based colorimetric assay (405-420 nm)	Plasma, urine, & other sample types	0.8-500 pg/ml	Most sensitive assay offered by Cayman
516360	8-Isoprostane Express ELISA Kit	Plate-based colorimetric assay (405-420 nm)	Plasma, urine, & other sample types	2.5-1,500 pg/ml	Results in <4 hrs
500431	STAT-8-Isoprostane ELISA Kit	Plate-based colorimetric assay (405-420 nm)	Plasma, urine, & other sample types	4.9-3,000 pg/ml	Results in <2.5 hrs

### **Lipid Peroxidation Probes**

Item No.	Product Name		Excitation (nm)	Emission (nm)	Key Features
27086	C11 BODIPY 581/591	Reduced:	<b>581</b>	<b>5</b> 91	A ratiometric fluorescent indicator of lipid oxidation
27000	C11 BODIP1 361/391	Oxidized:	<b>5</b> 00	<b>510</b>	A ratiometric nuorescent indicator or lipid oxidation
18798	MitoPerOx		<b>4</b> 95	■ 590 → ■ 520 shift	A ratiometric fluorescent probe for mitochondrial lipid peroxidation
62237	DPPP		<b>351</b>	<b>380</b>	A fluorescent probe for the detection of hydroperoxides

## **Clickable Tags**

Isolate and identify the reaction products of lipid peroxidation with these clickable tags available from Cayman.

Item No.	Product Name
13265	4-hydroxy Nonenal Alkyne
17104	4-oxo-2-Nonenal Alkyne

# Oxidized Phospholipid Lipidomic Services

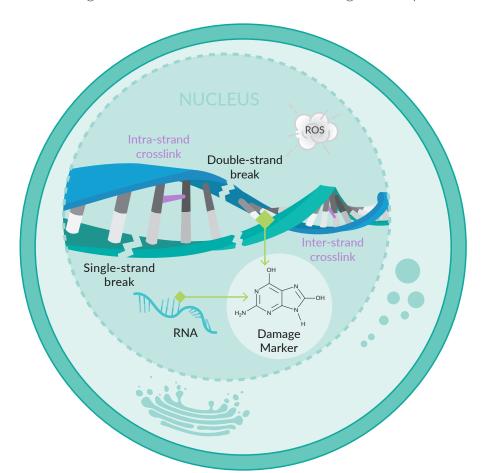
A targeted panel of phospholipids containing oxidized acyl chains (e.g., 20:4-OH, 20:4-OOH) will help identify hydroperoxy and hydroxy phospholipids in your samples.

www.caymanchem.com/lipidomics

13 Oxidative Stress: Markers & Detection Tools www.caymanchem.com

# **DNA & RNA Damage**

Oxidative stress can damage DNA and RNA, resulting in nucleobase lesions, double- and single-strand breaks, and inter- and intra-strand crosslinks, among other damage markers. Guanine is the base most prone to oxidation when DNA and RNA are damaged. The repair processes that are initiated to correct this damage result in the excretion of oxidized guanine species into the urine.



## **Damage Markers**

### 8-Hydroxyguanine

Item No. 89290

8-Hydroxyguanine is a marker of both DNA and RNA oxidative damage.

## 8-Hydroxyguanosine

Item No. 89300

8-Hydroxyguanosine is a marker of RNA damage.

## 8-Hydroxy-2'deoxyguanosine (8-OHdG)

Item No. 89320

8-Hydroxy-2'-deoxyguanosine is a marker of DNA damage.

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## **Tools to Measure DNA Damage**

# DNA/RNA Oxidative Damage (High Sensitivity) ELISA Kit

Item No. 589320

Measure the major oxidative damage markers 8-hydroxy-2'-deoxyguanosine, 8-hydroxyguanosine, and 8-hydroxyguanine in a variety of sample types.

# DNA/RNA Oxidative Damage (Clone 7E6.9) ELISA Kit

Item No. 501130

Measure the DNA oxidative damage marker 8-hydroxy-2'-deoxyguanosine and the RNA damage marker 8-hydroxyguanosine with equal selectivity and sensitivity.



Read our article to compare these assay kits side-by-side and choose the best one for your experiments at www.caymanchem.com/DNA-RNA-Damage

## **Complementary Assays**

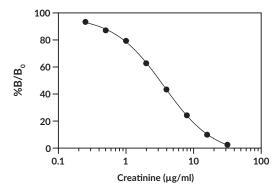
The concentration of urinary analytes may be impacted by hydration status. It is recommended that values obtained from urine samples be standardized to creatinine levels.

#### Creatinine ELISA Kit

Item No. 502330

#### Features:

- Measure creatinine in plasma, serum, and urine samples
- Assay 24 samples in triplicate or 36 samples in duplicate
- Lower limit of detection is 0.27 μg/ml
- Results in 2.5 hours

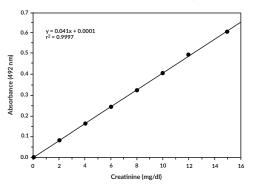


## Creatinine (urinary) Colorimetric Assay Kit

Item No. 500701

#### Features:

- · Measure creatinine levels in urine
- · Assay 40 samples in duplicate
- Measure creatinine levels down to 0.1 mg/dl
- Plate-based colorimetric measurement (490-500 nm)



#### **Reactive Probes & Antibodies**

Item No.	Product Name	Key Features
16952	4-Thiouracil	A photoactivatable probe used to detect RNA structures & nucleic acid-nucleic acid contacts
10009350	Aldehyde Reactive Probe (trifluoroacetate salt)	A biotinylated probe for detecting AP sites in damaged DNA
20094	DNA/RNA Oxidative Damage Monoclonal Antibody (Clone 7E6)	Host: Mouse · Reactivity: Species independent · Applications: Affinity purification, ELISA
25781	H2AX Phospho-Ser139 (1H2) Monoclonal Antibody	Host: Mouse · Reactivity: Human, mouse · Applications: ELISA, ICC, WB
32183	Histone H2AX (C-Term) Monoclonal Antibody	Host: Rabbit · Reactivity: Vertebrates · Applications: ELISA, ICC, multiplex assays, WB

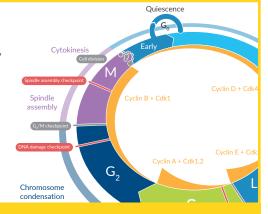
## Cell Cycle & Viability Assay Kits

Cellular detection of DNA damage can activate cell cycle checkpoints in  $\rm G_1$  phase, S phase, and at the  $\rm G_2/M$  transition to arrest the cell cycle and allow for DNA repair. Failure to repair DNA can force cells with damaged DNA to progress into mitosis, leading to mitotic catastrophe and cell death or senescence.

## Cell Cycle Phase Determination Kit - Item No. 10009349

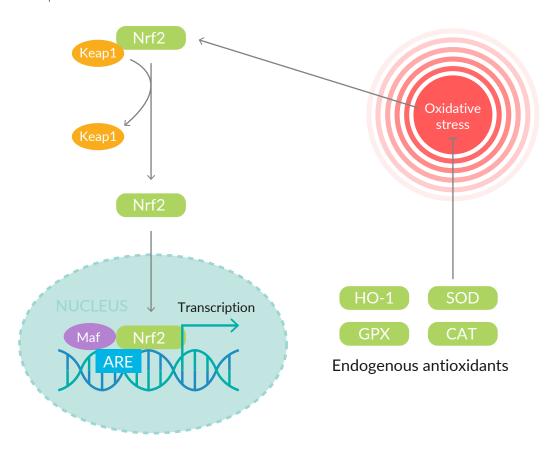
- Easy-to-use kit for flow cytometric analysis of cell cycle progression
- Determine the percentage of cells in  $G_0/G_1$ ,  $G_2$ , or S phase

Browse all Cell Health & Viability Assays at www.caymanchem.com



## **Antioxidants**

The expression of many enzymatic antioxidants is regulated by nuclear factor erythroid 2-related factor 2 (Nrf2). In normal conditions, Nrf2 is associated with the repressor protein Keap1. Under oxidative stress, Keap1 is degraded and Nrf2 translocates to the nucleus, where it binds to antioxidant response elements (AREs) in the promoter regions of many genes encoding antioxidant enzymes to enhance their expression.



#### **Enzymatic Antioxidant Assay Kits**

Item No.	Product Name	Format	Sample Types	Assay Range
707002	Catalase Assay Kit*	Plate-based colorimetric assay (540 nm)	Plasma, serum, tissue homogenates, erythrocyte & cell lysates	2-35 nmol/min/ml
703102	Glutathione Peroxidase Assay Kit	Plate-based colorimetric assay (340 nm)	Plasma, tissue homogenates, erythrocyte & cell lysates	50-344 nmol/min/ml
706002	Superoxide Dismutase Assay Kit	Plate-based colorimetric assay (440-460 nm)	Plasma, serum, tissue homogenates, & cell lysates	0.005-0.05 U/ml

<sup>\*</sup>Item No. 700910 is provided without H<sub>2</sub>O<sub>2</sub> to our international customers with shipping restrictions

## Nrf2 Transcription Factor Assay Kit

Item No. 600590

 A sensitive, non-radioactive method of detecting Nrf2 from whole cell lysates

#### **SOD Mimetics**

Item No.	Product Name	Key Features
31112	Imisopasem Manganese	A non-peptide SOD mimetic
18796	MitoTEMPOL	A mitochondrial-targeting SOD mimetic
27051	TEMPOL	A spin label & SOD mimetic

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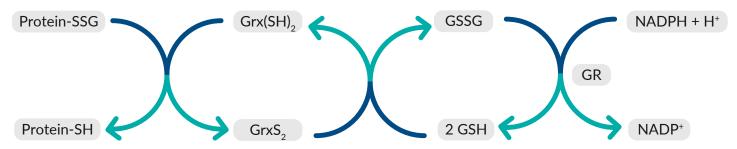
www.caymanchem.com Oxidative Stress: Markers & Detection Tools

## Glutaredoxin & Thioredoxin

The glutaredoxin (Grx) and thioredoxin (Trx) systems balance cellular redox status.

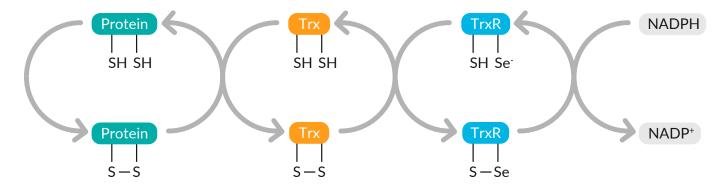
## **Glutaredoxin System**

Grx catalyzes the deglutathionylation of glutathionylated proteins and the reduction of protein disulfides. Oxidized  $Grx (GrxS_2)$  is reduced  $(Grx(SH)_2)$  by glutathione (GSH), which produces GSSG, the oxidized form of GSH. GSSG is reduced back to GSH by glutathione reductase (GR) at the expense of NADPH. The GSH:GSSG ratio is often used as a marker of oxidative stress.



## **Thioredoxin System**

Trx is responsible for the reduction of protein disulfides. Upon reduction of oxidized protein disulfides, Trx is oxidized. Oxidized Trx  $(Trx(S_2))$  is restored to its reduced state by thioredoxin reductase (TrxR) at the expense of NADPH.



#### **Enzymatic Antioxidant Assay Kits**

Item No.	Product Name	Format	Sample Types	Assay Range
703002	Glutathione Assay Kit	Plate-based colorimetric assay (405-414 nm)	Plasma, serum, cell & erythrocyte lysates, & tissue homogenates	0.5-16 μM (GSH) or 0.25-8 μM (GSSG)
500239	Glutaredoxin Fluorometric Activity Assay Kit	Plate-based fluorometric assay (Ex/Em = 520/560 nm)	Cell lysates & tissue samples	0.066 ± 0.013 μM/min
703202	Glutathione Reductase Assay Kit	Plate-based colorimetric assay (340 nm)	Plasma, cell & erythrocyte lysates, & tissue homogenates	20 U/ml
500228	Thioredoxin Fluorometric Activity Assay Kit	Plate-based fluorometric assay (Ex/Em = 520/560 nm)	Cell lysates	0.01 ± 0.002 μM/min
10007892	Thioredoxin Reductase Colorimetric Assay Kit*	Plate-based colorimetric assay (405-414 nm)	Tissue homogenates & cell lysates	0.08 U/ml

\*Item No. 11529 is available as a fluorometric alternative

#### **Natural Product Antioxidants**

Item No.	Product Name	Key Features
70935	(–)-Epigallocatechin Gallate	A phenol with diverse biological activities
26758	(±)-β-Tocopherol	A form of vitamin E
10004235	cis-Resveratrol	A phenolic antioxidant in red wine
14656	L-Ascorbic Acid	A reducing agent
70945	Lycopene	A natural carotenoid
20261	N-acetyl-L-Cysteine	An antioxidant & GSH precursor
70675	trans-Resveratrol	A polyphenol with diverse biological activities
10008377	α-Tocotrienol	An antioxidant with neuroprotective properties
16837	β-Carotene	An antioxidant & vitamin A precursor

View additional natural product antioxidants online at www.caymanchem.com

#### **Synthetic Antioxidants**

Item No.	Product Name	Key Features
89910	BHT	A synthetic antioxidant
17730	Liproxstatin-1	An inhibitor of lipid peroxidation
89950	Mitoquinol	A mitochondria-targeted antioxidant
10011659	Trolox	A vitamin E derivative

#### **Antioxidant Activity Probes**

Item No.	Product Name	Key Features
27317	ABTS (ammonium salt)	Used to assess antioxidant capacity in the Trolox equivalent antioxidant capacity (TEAC) assay
14805	DPPH	A colorimetric probe (515 nm) for free radical scavengers
27089	STY-BODIPY	An indicator of radical- trapping antioxidant activity

## **Assay Kits for Antioxidants**

### Antioxidant Assay Kit - Item No. 709001

- Measure the total antioxidant capacity of a variety of sample types in Trolox equivalents
- Plate-based colorimetric assay (405 or 750 nm)

### Ascorbate Assay Kit - Item No. 700420

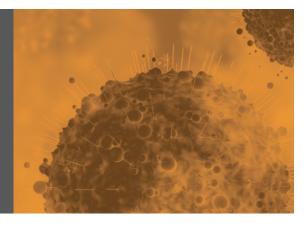
- Quantify ascorbate from plasma, serum, urine, & fruit juice
- Plate-based fluorometric assay (Ex/Em = 340-350/420-430 nm)

# Cell Death Mechanisms & Detection Tools Guide

Assess the ultimate consequence of oxidative injury with a cell death assay. This guide can help you determine the cell death mechanism(s) occurring in your experiment.

www.caymanchem.com/celldeath

**DOWNLOAD THE GUIDE** 



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www.caymanchem.com Oxidative Stress: Markers & Detection Tools



1180 East Ellsworth Road Ann Arbor, MI 48108 www.caymanchem.com

## Phone:

(800) 364-9897 (toll free)

## Fax:

(734) 971-3640

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