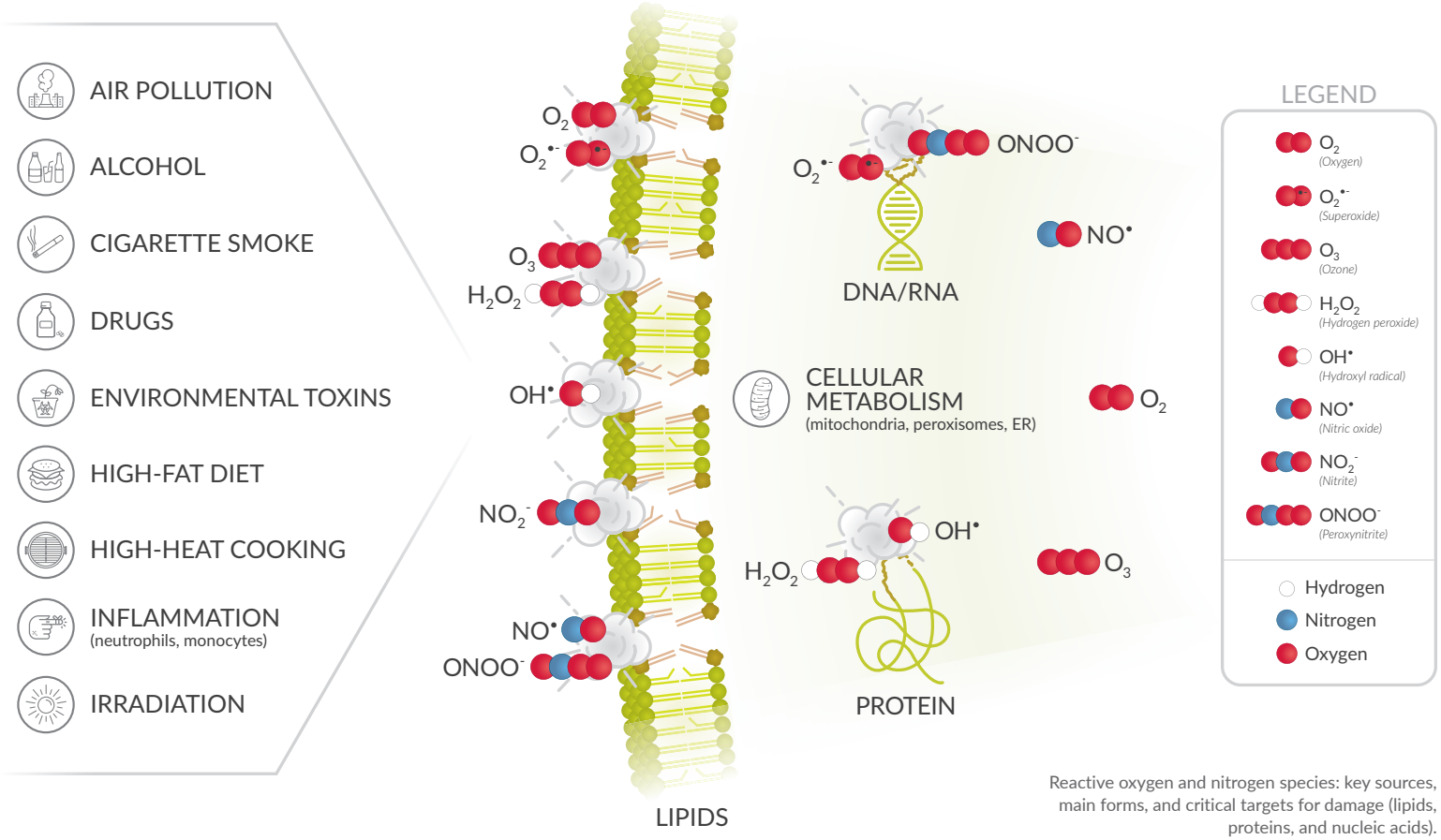
Stressed about Picking an Oxidative Damage Assay?

A Guide to Finding the Right Biomarker to Detect in Your Application

Oxidative stress can be evaluated directly by measuring reactive oxygen species (ROS) or indirectly by the associated damage to lipids, proteins, and nucleic acids that occurs upon overproduction of ROS. Although direct measurement of ROS is ideal, the indirect methods are often relied on more heavily due to the relative stability of damage markers on biomolecules compared to the transient nature of ROS. To help you determine which is best to use in your experimental system, here is a breakdown of the assay technology used to detect the most common oxidative damage biomarkers.



Reactive oxygen and nitrogen species: key sources, main forms, and critical targets for damage (lipids, proteins, and nucleic acids).

ROS

Oxygen is electronically reduced as part of normal metabolism, resulting in the formation of various ROS, including hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\bullet-}$). Damage to cellular macromolecules occurs when uncontrolled oxidation stresses a biological system. Assays for ROS do not discern the source of ROS production (*i.e.*, normal *versus* disease state), but if the experimental model is under stress, an increase in ROS and alteration to molecular components is probable.

H_2O_2

H_2O_2 can be detected using sensitive probes such as ADHP coupled to an enzyme like HRP. Assay specificity is improved significantly when an H_2O_2 scavenger, such as catalase, is included as a control.

Dihydroethidium

Dihydroethidium (hydroethidine or DHE) can be used directly in live cells. This redox-sensitive probe is oxidized by $O_2^{\bullet-}$ to form 2-hydroxyethidium (ex 500-530 nm/em 590-620 nm) or by non-specific oxidation by H_2O_2 or other sources of ROS to form ethidium (ex 480 nm/em 576 nm).

Xanthine Oxidase

Xanthine oxidase (XO) produces both H_2O_2 and $O_2^{\bullet-}$. The activity of this enzyme can be measured by allowing XO to degrade hypoxanthine and capturing the H_2O_2 byproduct of this reaction *via* a probe like ADHP coupled to an enzyme like HRP.

See ROS detection assays on page 3.

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RNS

Reactive nitrogen species (RNS) are also produced during oxidative stress. High levels of nitric oxide ($NO^{\bullet}$), synthesized by nitric oxide synthase (NOS), and $O_2^{\bullet-}$ lead to the formation of peroxynitrite. $NO^{\bullet}$ itself also reacts with thiols and iron-sulfur enzymes, whereas peroxynitrite reacts with tyrosine residues to form nitrotyrosine.

Nitrate (NO_3^-)/Nitrite (NO_2^-)

NO_3^- and NO_2^- are end products of *in vivo* $NO^{\bullet}$ reactions whose total production can be detected using either Griess reagents or DAN. These assays first convert NO_3^- to NO_2^- using NADPH-dependent nitrate reductase. Subsequent reaction with Griess reagents or DAN, both of which only react with NO_2^-, will determine a total concentration of NO_2^-.

NOS

NOS activity is detected in tissues and cells by harnessing the NOS-driven conversion of a radio-labeled arginine to citrulline in the presence of the necessary factors. Alternatively, *in vitro* NOS activity can be detected using the chemistry of the Griess reaction once the excess NADPH added as a cofactor for NOS activity is removed using an oxidization step that is catalyzed by lactate dehydrogenase.

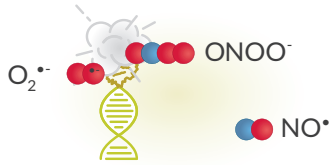
See RNS detection assays on page 3.

DNA/RNA Damage

Guanine is the base that is most prone to oxidation when DNA and RNA are damaged. The repair processes that are initiated to correct this damage release the following oxidized guanine species into the urine:

- 8-hydroxyguanine — the ribose-free base
- 8-hydroxyguanosine — the nucleoside from RNA
- 8-hydroxy-2'-deoxyguanosine — the deoxynucleoside from DNA

Assays that can detect multiple oxidized guanine species capture a more complete set of biologically relevant products of oxidative damage than do assays that are restricted to analysis of only one (*e.g.*, 8-hydroxy-2'-deoxyguanosine).

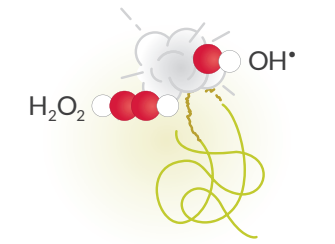


DNA/RNA damage. *Note that the $O_2^{\bullet-}$ and $ONOO^-$ molecules are representative ROS and RNS species that cause damage. Many additional species can damage DNA and RNA as well.*

See DNA/RNA damage detection assays on page 4.

Protein Oxidation and Nitration

The most common marker of protein oxidation is protein carbonyl content. Redox cycling cations bind to proteins and, in conjunction with attack by ROS, lead to the formation of amino acid derivatives containing carbonyl groups (ketones, aldehydes). Cigarette smoke and aldehydes also introduce carbonyls into proteins. Alternatively, ROS exposure to a protein's methionine residues generates protein methionine sulfoxide (MetO), an oxidative modification, that if not reversed by MetO reductases is further oxidized to methionine sulfone and can lead to protein dysfunction. The presence of nitrotyrosine on proteins is used as a marker of peroxynitrite formed *in vivo* when $NO^{\bullet}$ reacts with $O_2^{\bullet-}$. As peroxynitrite undergoes heterolytic cleavage, freed nitronium ions nitrate protein tyrosine residues. $NO^{\bullet}$ can directly modify proteins through the RNS-mediated process of S-nitrosylation wherein an $NO^{\bullet}$ group binds to thiol groups of protein cysteine residues resulting in the formation of an S-NO moiety.



Protein damage. *Note that the H_2O_2 and $OH^{\bullet}$ molecules are representative ROS species that cause damage. Many additional species can damage proteins as well.*

Carbonyl Content

A convenient technique to detect carbonyl content in protein preparations involves reaction between DNPH and protein carbonyls, which forms a Schiff base that produces a corresponding hydrazone that can be measured spectrophotometrically.

Methionine Sulfoxide (MetO)

An antibody specific for protein MetO is used to monitor oxidative modifications by detecting proteins containing MetO residues.

Nitrotyrosine

An antibody specific for nitrotyrosine is used to detect protein nitration.

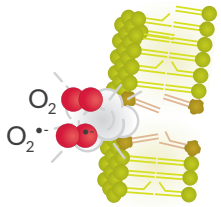
S-Nitrosylation

Protein S-nitrosylation can be directly visualized using the biotin switch technique. This method cleaves S-NO bonds (after blocking existing free thiols) to biotinylate the resulting newly formed free thiol groups.

See protein oxidation and nitration detection assays on page 4.

Lipid Peroxidation

Lipid peroxidation results in the formation of highly reactive and unstable hydroperoxides of unsaturated lipids. They can be measured either directly or assessed indirectly by the various decomposition products (*e.g.*, alkanes, ketones, aldehydes) of unstable hydroperoxides. 8-Isoprostane, produced by random oxidation of tissue phospholipids, is currently considered one of the most reliable biomarkers of *in vivo* lipid peroxidation. It is a specific product of lipid peroxidation that is stable and levels are present in detectable quantities in all normal biological fluids and tissues. Measurement of levels esterified in phospholipids can be used to determine the extent of lipid peroxidation in target sites of interest.



Lipid damage. *Note that the O_2 and $O_2^{\bullet-}$ molecules are representative ROS species that cause damage. Many additional species can damage lipids as well.*

Lipid Hydroperoxides (LPOs)

LPOs can be efficiently extracted and measured directly by utilizing redox reactions with ferrous ions to reveal the total hydroperoxide content present at a moment in time.

4-hydroxy Nonenal (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.

Malondialdehyde (MDA)

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

8-Isoprostane

8-Isoprostane is typically assessed using either an immunoassay or LC-MS or GC-MS.

See lipid peroxidation detection assays on page 4.

Read the complete guide to **oxidative damage kits** at
www.caymanchem.com/oxidativedamage

Kit Recommendations

ROS Assays

Item No.	Product Name	Measure	Additional Info
600050	Hydrogen Peroxide Cell-Based Assay Kit	Extracellular H_2O_2	Utilizes ADHP, a sensitive and stable probe for H_2O_2, and includes catalase to check assay specificity
601290	ROS Detection Cell-Based Assay Kit (DHE)	ROS	Utilizes the redox-sensitive probe DHE as a substrate for $O_2^{\bullet-}$ and H_2O_2; includes positive control for ROS generation and negative control for ROS scavenging
10010895	Xanthine Oxidase Fluorometric Assay Kit	XO activity	Based on a multistep enzymatic reaction in which the H_2O_2 produced when XO oxidizes hypoxanthine reacts with ADHP

RNS Assays

Item No.	Product Name	Measure	Additional Info
780001	Nitrate/Nitrite Colorimetric Assay Kit	NO• metabolites	Uses a small amount of added NADPH in conjunction with a catalytic system for recycling spent $NADP^+$ back to NADPH to avoid NADPH interference with the chemistry of the Griess reagents; works well for the analysis of fluids such as plasma and urine, but cannot be used to analyze NO_2^- and NO_3^- from an *in vitro* assay of NOS in which excess NADPH has been added
760871	Nitrate/Nitrite Colorimetric Assay Kit (LDH method)	*In vitro* NOS activity and NO• metabolites	Use to analyze NO_2^- and NO_3^- from an *in vitro* NOS assay in which excess NADPH has been added; an extra step is included in the protocol that uses LDH to remove the excess NADPH
780051	Nitrate/Nitrite Fluorometric Assay Kit	NO• metabolites	Utilizes DAN instead of Griess reagents, which enables 20-fold increased sensitivity over the colorimetric version; allows for detection of low concentrations of NO_2^- and NO_3^- (minimum detectable quantity of NO_2/NO_3 is ~50 nM)
781001	NOS Activity Assay Kit	NOS activity	Monitors the conversion of radiolabeled arginine to citrulline by NOS

DNA/RNA Damage Assays

Item No.	Product Name	Measure	Additional Info
589320	DNA/RNA Oxidative Damage ELISA Kit	8-hydroxy-2'-Deoxyguanosine, 8-hydroxyguanosine, and 8-hydroxyguanine	Monoclonal antibody (clone 15A3) enables detection of 8-hydroxy-2'-deoxyguanosine (DNA oxidative damage marker), 8-hydroxyguanosine (RNA damage marker), and 8-hydroxyguanine (DNA/RNA damage marker) with selectivity and sensitivity highest for 8-hydroxy-2'-deoxyguanosine; does not correlate with LC/MS measurements of 8-hydroxy-2'-deoxyguanosine because the ELISA also detects 8-hydroxyguanosine and 8-hydroxyguanine
501130	DNA/RNA Oxidative Damage (Clone 7E6.9) ELISA Kit	8-hydroxy-2'-Deoxyguanosine and 8-hydroxyguanosine	Monoclonal antibody (clone 7E6.9) enables detection of 8-hydroxy-2'-deoxyguanosine (DNA oxidative damage marker) and 8-hydroxyguanosine (RNA damage marker) with equal selectivity and sensitivity; correlates with LC/MS measurements of a combination of 8-hydroxy-2'-deoxyguanosine and 8-hydroxyguanosine

Protein Oxidation and Nitration Assays

Item No.	Product Name	Measure	Additional Info
10005020	Protein Carbonyl Colorimetric Assay Kit	Protein carbonyl content	Utilizes the reaction between DNPH and protein carbonyls as a readout of protein oxidation
600160	Methionine Sulfoxide Immunoblotting Kit	Proteins containing MetO residues	Utilizes a MetO polyclonal antibody isolated from rabbit serum that is specific for MetO and demonstrates minimal cross reactivity with methionine sulfone
601220	Nitrotyrosine IP Kit ***Also available:*** *Nitrotyrosine EIA Antiserum (Item No. 489542) and Nitrotyrosine AChE tracer (Item No. 489540)*	Nitrated tyrosine content	Utilizes a sorbent coupled with a nitrotyrosine monoclonal antibody to capture and pulldown nitrated proteins
10006518	S-Nitrosylated Protein Detection Kit (Biotin Switch)	S-NO proteins	Utilizes a modified 'Biotin-switch' method to directly tag S-NO proteins
10010721	S-Glutathionylated Protein Detection Kit	Protein-PSSG adducts	Utilizes a modified 'Biotin-switch' method to directly tag protein-PSSG adducts

Lipid Peroxidation

Item No.	Product Name	Measure	Additional Info
501140	DHN-MA EIA Kit	DHN-MA, a 4-HNE metabolite	Compatible with human, mouse, rat, dog, and pig samples
516351	8-Isoprostane ELISA Kit	8-Isoprostane	Overnight assay (incubation time = 18 hours) uses AChE tracer; assay range 0.8-500 pg/ml; detection limit (80% B/B_0) of ~3 pg/ml
516360	8-Isoprostane Express ELISA Kit	8-Isoprostane	4 hour assay uses AChE tracer; assay range 2.5-1,500 pg/ml; detection limit (80% B/B_0) of ~10 pg/ml
500431	STAT-8-Isoprostane ELISA Kit	8-Isoprostane	Extremely rapid assay (results in ~2 hours) uses an AP tracer; assay range 23.4-3,000 pg/ml; detection limit (80% B/B_0) of ~45 pg/ml
705002	Lipid Hydroperoxide (LPO) Assay Kit	LPOs	Designed for use with a single-tube spectrophotometer to read the results
705003	Lipid Hydroperoxide (LPO) Assay Kit (96 well)	LPOs	Designed for use with a reusable glass plate
10009055	TBARS Assay Kit	MDA-TBA adduct	Standard method to determine lipid peroxidation; reaction yields higher sensitivity when measured fluorometrically, but a colorimetric method is included as an option
700870	TBARS (TCA Method) Assay Kit	MDA-TBA adduct	Offers the advantage of improved sample processing and reduced working volumes by incorporating a TCA precipitation procedure; maintains same reliability and accuracy as the original TBARS Assay; includes sample acid precipitation protocol to avoid confounding soluble TBARS

View a complete list of our **nitric oxide & oxidative injury assay kits** at **www.caymanchem.com**