ISSUE 23.1

CAYMAN CURRENTS

Cayman CHEMICAL

REDOX SIGNALING

IN THIS ISSUE:

Chemical Probes for Detecting Endogenous Protein *S*-Nitrosation

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Cysteine oxidation by the gaseous second messenger nitric oxide leads to the reversible modification of select cysteine residues in proteins, termed either *S*-nitrosylation or *S*-nitrosation.[1] This modification is non-enzymatic and relies largely on a protein's proximity to diffusible nitric oxide. Just as nitric oxide levels are tightly regulated by hormone-sensitive nitric oxide synthases, it follows that *S*-nitrosation can also be hormonally regulated. Indeed, inducible nitric oxide synthase stimulates the *S*-nitrosation of interacting proteins, which in turn exchange *S*-nitroso groups and transfer the modification to select sites on target proteins.[2] In hundreds of examples, *S*-nitrosation can modulate the function of cellular proteins, often by transient inactivation of functional cysteines in enzyme active sites. Classes of enzymes affected include metabolic enzymes, cysteine proteases, phosphatases, and ion channels.[3] Since active site cysteines have lower pK_a values, they are more nucleophilic and more readily react with cellular electrophiles. Therefore, *S*-nitrosation and other oxidants favor functional cysteines, leading to direct modulation of cellular pathways.[4] Solvent accessible *S*-nitrosated cysteines are readily exchanged with reduced glutathione, which then delivers *S*-nitrosoglutathione for further enzymatic reduction. In contrast, *S*-nitrosation also occurs on cysteines in protected protein environments, such as in the active site of enzymes. In this hindered environment, *S*-nitrosation can be long-lived and functionally block protein function to disrupt cellular pathways.

Given the broad role of *S*-nitrosation in cellular regulation, it is important to have robust, selective tools for biochemical analysis.[5] Interest in this research area has fueled the development of chemical enrichment strategies to purify and analyze protein *S*-nitrosation, including ascorbate-dependent switch techniques, organomercury enrichment, phosphine ligations, and sulfinic acid catalyzed thiosulfonate formation.[6-13] All of these methods require complete alkylation of cellular thiols to prevent disulfide scrambling and exchange of the probe-linked adducts.

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Biotin Switch Technique (BST)

The primary method to biochemically characterize protein *S*-nitrosation is termed the biotin switch technique or BST (**Figure 1**).[6,7] In this approach, cell lysates are first denatured in a buffered detergent solution supplemented with the metal chelator neocuproine, which prevents copper-catalyzed breakdown of *S*-nitrosothiols. *S*-Nitrosothiols are particularly light sensitive, so it is critical to avoid exposure to daylight to prevent photolysis and disulfide scrambling. After denaturation, the sample is treated with the cysteine alkylation agent methyl methane thiosulfonate (MMTS), which reacts with free thiols to form inert disulfides, releasing methylsulfinic acid. Next, the sample is incubated with ascorbate, which selectively reduces nitrosothiols to thiols without affecting disulfides. Importantly, cysteine sulfenic acids are especially unstable on denatured proteins and alkylated after denaturation to eliminate any cross reactivity.[11] After ascorbate reduction, any newly reduced thiols can react with either thiopropyl sepharose or biotin-HPDP for affinity enrichment and biochemical analysis. After resin enrichment, proteins are eluted by reduction of the disulfide-resin linkage and quantified by either Western blot or mass spectrometry. The biotin switch technique is thoroughly validated and widely used for indirect analysis of endogenous protein *S*-nitrosation.

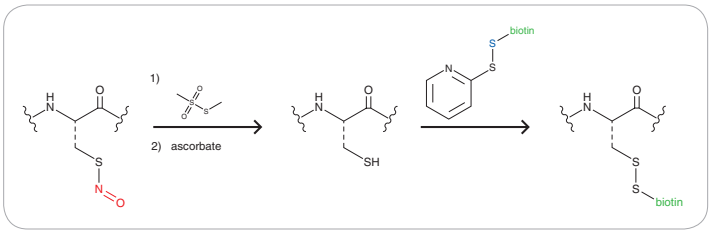

Figure 1. Biotin Switch Technique (BST)

Organomercury Enrichment

More recently, several other chemoselective methods have emerged for *S*-nitrosothiol conjugation. Organomercury reagents react with thiols and nitrosothiols to form a stable mercury-thiolate conjugate (**Figure 2**).[8] After complete alkylation of cellular thiols, organomercury resin or biotin-mercury conjugates allow direct enrichment of *S*-nitrosated proteins with no cross reactivity with disulfides. The mercury-linked proteins are then oxidized with performic acid to release modified proteins as observed with oxidized sulfonic acids. Since these probes are not commercially available, this method requires synthetic efforts using toxic mercury, warranting extreme safety precautions. Nonetheless, organomercury enrichment provides a highly sensitive and in-depth mass spectrometry profile of endogenous protein *S*-nitrosation from mouse tissues, allowing the identification of hundreds of endogenous target proteins and sites of *S*-nitrosation.[14]

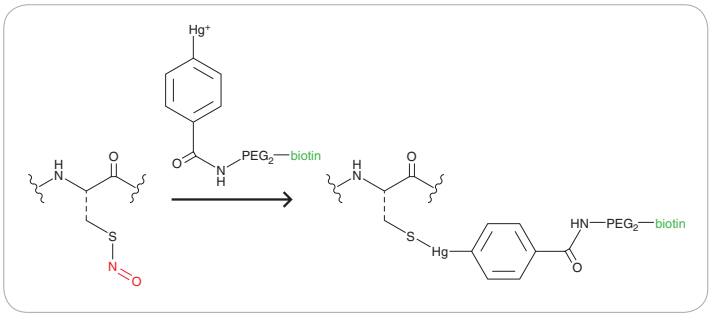

Figure 2. Organomercury Enrichment

Phosphine Ligations

Organophosphine reagents have also been reported as robust probes for selective nitrosothiol conjugation (**Figure 3**).[5,9,10] Triarylphosphine probes lead to the chemo-selective reduction of *S*-nitrosothiols, initiated by the reaction of phosphine with the *S*-nitroso group to form an aza-ylide. Triarylphosphine probes have been designed to undergo a series of concerted reactions to yield different chemical linkages, including the bis-ligation with a triarylphosphine-thioester probe to yield a disulfide-iminophosphorane product.[10] This probe is unique since it provides a direct linkage to both the originating nitric oxide nitrogen and the cysteine sulfur. While this approach was initially reported to detect *S*-nitrosated metabolites by mass spectrometry, it was later reported to achieve poor ionization efficiency in mass spectrometry-based proteomics analyses.[15,16] A separate triarylphosphine probe yields the one-step formation of a disulfide linkage at the site of *S*-nitrosation.[9] Here the phosphine-thioester first forms a thiobenzamide adduct and thiolate followed by intermolecular thioester exchange with the released thiolates to generate a disulfide linkage. Altogether, SNO trapping by triarylphosphine (SNO-trap) methods enable robust enrichment and mass spectrometry profiling of native sites of *S*-nitrosation. Indeed, SNO-trap methods were used to identify differentially *S*-nitrosated proteins in a mouse model of Parkinson's disease, revealing direct links to disease pathology.[16]

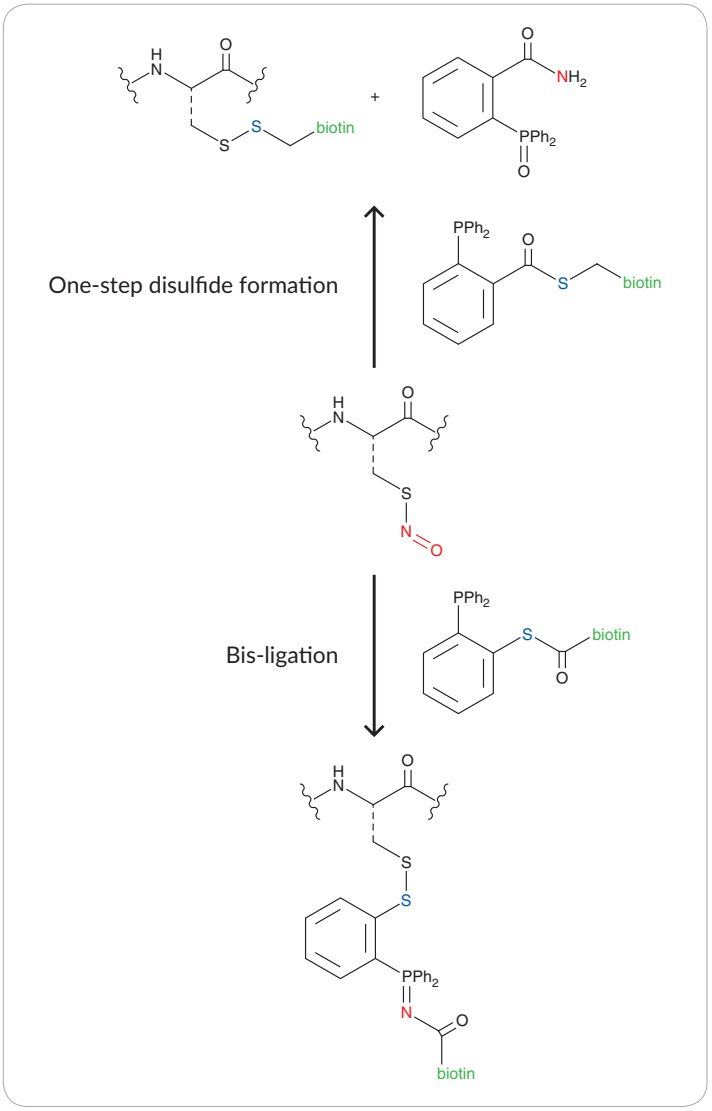

Figure 3. Phosphine Ligations

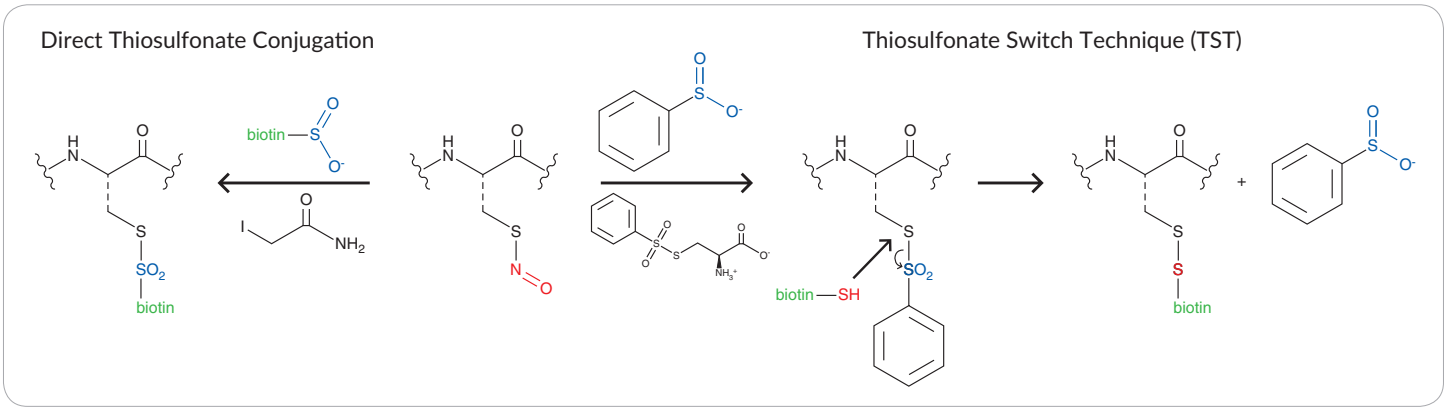

Figure 4. Sulfinic Acid Conjugation

Sulfinic Acid Conjugation

S-Nitrosocysteine was first reported to react with phenylsulfinic acid to form a thiosulfonate linkage more than 30 years ago.[17] Based on this reactivity, sulfinic acid probes have now been shown to modify sites of endogenous *S*-nitrosation on proteins, either by direct enrichment with biotinylated sulfinic acids or indirectly by exchange of the resulting thiosulfonate with a thiol-linked probe (**Figure 4**).[11,13] Sulfinic acids are unreactive towards cystine, oxidized glutathione, and the activated disulfide in Ellman's reagent.[11] Furthermore, phenylsulfinic acid does not react with sulfenamides, which equilibrate to sulfenic acids in solution. Therefore, sulfinic acids exhibit privileged reactivity with *S*-nitrosothiols, yielding a stable thiosulfonate linkage. Similar to MMTS, thiosulfonates are electrophilic and react with free thiols to release methylsulfinic acid. In the thiosulfonate switch technique (TST), lysates are first alkylated with *S*-phenylsulfonylcysteine, which releases phenylsulfinic acid.[13] Next, additional phenylsulfinic acid is added to the lysate, which converts nitrosothiols to thiosulfonates in acidic buffers. Finally, a thiol-linked biotin or fluorophore is added, which under acidic conditions reacts preferentially with thiosulfonates over any disulfides, yielding a more stable disulfide linkage at the former site of *S*-nitrosation.

Conversely, *S*-nitrosated proteins can be directly conjugated to a probe in one step with biotin-conjugated sulfinic acids, such as the metabolite hypotaurine (biotin-SO_2H).[11] After complete alkylation with iodoacetamide, the lysate is incubated with biotin-SO_2H forming a thiosulfonate linkage at sites of endogenous *S*-nitrosation. Thiosulfonates are stable at neutral pH, providing a direct linkage for gel-based or mass spectrometry quantitation of endogenous *S*-nitrosation. Interestingly, MMTS alkylation prevents biotin-SO_2H conjugation, suggesting that methylsulfinic acid released by MMTS after thiol conjugation is present at sufficient concentrations to react at sites of *S*-nitrosation. While further studies are needed to explore this finding, it suggests that the ascorbate-dependent biotin switch technique may similarly lead to thiosulfonate formation after MMTS alkylation, which is then reduced by ascorbate. Finally, this reaction can be inverted to detect endogenous sulfinic acids on proteins, establishing a direct 1:1 stoichiometry in the reaction mechanism.[11] Overall, sulfinic acids and nitrosothiols provide a distinct biocompatible approach to profile the reciprocal reactivity of *S*-nitrosation and *S*-sulfination in biology.

Radical species are an unavoidable consequence of respiration and the environment and are tightly buffered by small molecule antioxidants and redox detoxifying enzymes. Aberrant oxidative signaling is perhaps one of the most important factors contributing to aging, neurodegeneration, heart disease, diabetes, and cancer. Now with several chemically orthogonal techniques, protein *S*-nitrosation can be readily analyzed by indirect detection of ascorbate-sensitive thiols or by direct conjugation to organomercury, sulfinic acids, or triarylphosphine probes. The variety of *S*-nitrosation detection methods demonstrates the unique chemical properties of this reversible, electrophilic protein modification. With this emerging *S*-nitrosation toolset, multiple techniques can be simultaneously applied to corroborate endogenous *S*-nitrosation on select proteins by comparative proteomic profiling.

References

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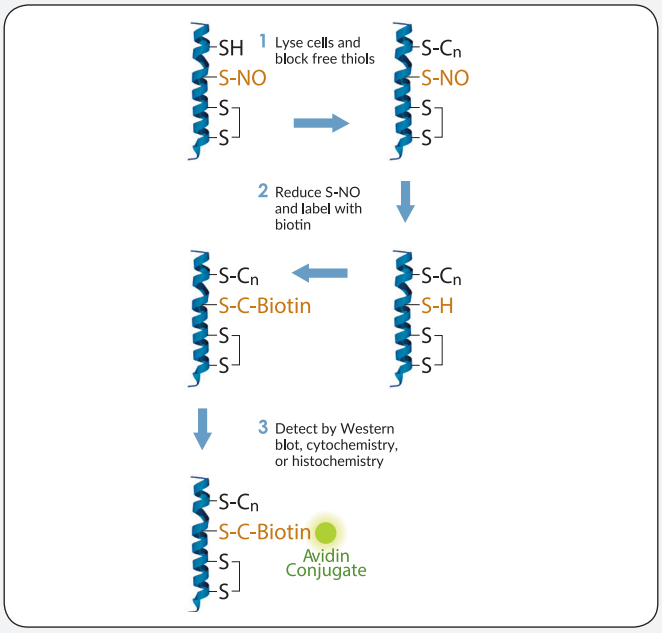
Thiol Modification Detection

Protein S-nitrosothiol formation depends upon several factors including thiol accessibility, protein conformation, and concentration of nitric oxide. To visualize protein S-nitrosothiol formation directly in whole cells or tissues or by Western blot analysis, Cayman offers two kit detection methods: a modified biotin switch technique (BST) and thiosulfonate switch technique (TST). Each kit provides all needed reagents to detect S-nitrosocysteine post-translational modifications by tagging free thiols with either maleimide-biotin or a rhodamine dye. The S-Nitrosothiol Assay Kit (Thiosulfonate Switch) is an optimized assay developed to circumvent limitations of the BST. The TST offers same day results and can be used as an orthogonal assay to the traditional BST.

Protein S-Nitrosothiol Detection

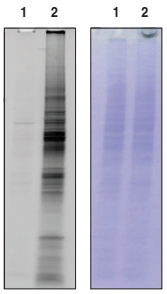
S-Nitrosylated Protein Detection Kit (Biotin Switch) 10006518

- A modified BST assay
- Direct visualization of S-NO proteins in whole cells or tissues
- Colorimetric or fluorometric detection



S-Nitrosothiol Assay Kit (Thiosulfonate Switch) 601210

- Sample preparation for SDS-PAGE in under four hours
- Detect endogenous or NO donor generated protein S-nitrosothiols
- Identify proteins with S-nitrosothiols by fluorescence immediately after electrophoresis
- Generate an in-gel standard curve for relative quantitation
- Prepare samples by TST and proceed directly to proteomic analysis



In-gel detection of protein S-nitrosothiols. Equal parts lysate were treated with vehicle (1) or NO donor (2) respectively, desalted, and treated with TST assay reagents according to the assay protocol. Left side image is TST rhodamine fluorescence and the right side image is the same gel post Coomassie Blue destaining. Image generated by UVP imager; four second capture with ethidium bromide filter, coupled with UV light source.

Modified Biotin Switch Technique

SH-**R**-SNO $\xrightarrow[\text{20 min., 37°C}]{\text{1) NEM, pH 8}}$ NEMS-**R**-SNO $\xrightarrow[\text{1-3 hr., >25°C}]{\text{Ascorbate}}$ NEMS-**R**-SH $\xrightarrow[\text{1 hr.}]{\text{Biocytin}}$ NEMS-**R**-S-biotin

2) Desalt (or pptn. 1 hr.)

Thiosulfonate Switch Technique

SH-**R**-SNO $\xrightarrow[\text{1 hr., 25°C}]{\text{SPSC, pH 4}}$ CysSS-**R**-SNO $\xrightarrow[\text{30 min., 25°C}]{\text{NaPhSO}_2}$ CysSS-**R**-SSO$_2$Ph $\xrightarrow[\text{10 min., 25°C}]{\text{Z-Rhodamine-SH}}$ CysSS-**R**-SS-ZRhodamine

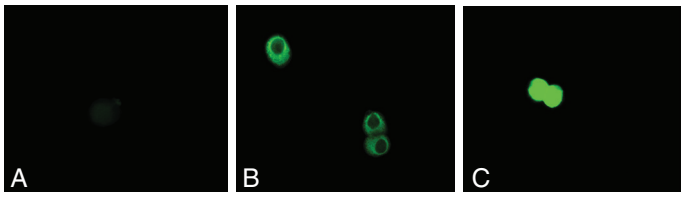
Which Kit is Right for You?

Assay Kit	pH	Conditions	Run Time	Detection	Notes
S-Nitrosylated Protein Detection Kit (Biotin Switch)	7.6	May require heating	1.5 days	Colorimetric or fluorometric	
S-Nitrosothiol Assay Kit (Thiosulfonate Switch)	4	Room temperature	<4 hours	Fluorometric	Includes a BSA rhodamine mixed disulfide positive control

Protein S-Glutathione Detection

S-Glutathionylated Protein Detection Kit 10010721

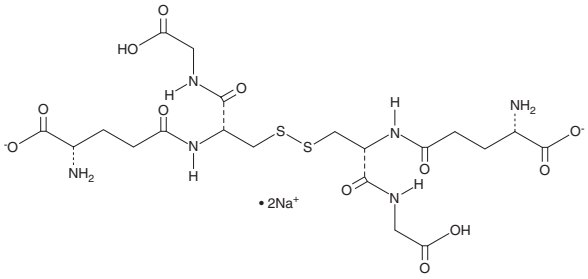
- Direct visualization of S-glutathionylated proteins in whole (permeabilized) cells by flow cytometry and microscopy
- Visualization using colorimetric or fluorescence detection
- Reagents provided to test up to 30 samples



Typical fluorescence images using 10,000 mouse monocytes per sample. *Panel A:* Cells stained by the standard method with omission of Reduction Reagent generated no fluorescence. *Panel B:* Cells stained by the method as written reveal S-glutathionylated proteins. *Panel C:* Cells treated by the method with omission of free-thiol Blocking Reagent reveals labeling of all accessible protein thiols.

L-Glutathione, oxidized (sodium salt) 15491

A hydrogen acceptor in the enzymatic determination of $NADP^+$ and NADPH and can be a proximal donor in S-glutathionylation post-translational modifications

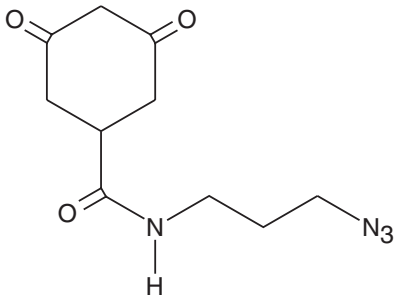


Also Available: L-Glutathione, reduced (Item No. 10007461)

Sulfenic Acid Probes

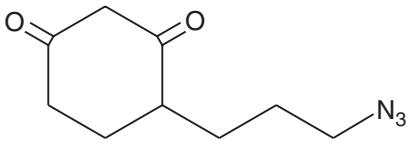
DAz-1 13173

A cell-permeable chemical probe that reacts specifically with sulfenic acid-modified proteins; this probe is less sensitive for sulfenic acid detection compared to its analog DAz-2



DAz-2 13382

A cell-permeable chemical probe that reacts specifically with sulfenic acid-modified proteins; azido group of DAz-2 provides a method for the selective conjugation to phosphine- or alkynyl-derivatized reagents, such as biotin or various fluorophores, for subsequent analysis of the labeled proteins



Redox Biology

In partnership with the IMCO Corporation of Sweden, Cayman offers several convenient methods for studying thioredoxin (Trx) and glutaredoxin (Grx) systems. Active and pure recombinant enzymes, validated antibodies, and assays are available to researchers worldwide. Unlike other commercially available products, the Trx and Grx assays come supplied with active, biologically relevant enzymes. These enzymes are paired with high-quality substrates to provide researchers with ready-to-use activity assays. These purified, active enzymes, as well as corresponding antibodies, can also be purchased separately to complete your tool kit for studying oxidative stress and cellular redox systems.

Redox Activity Assays

Item No.	Product Name	Summary
11536	Glutaredoxin Fluorescent Activity Assay Kit	Measure glutaredoxin activity in cell lysates and tissue homogenates using a fluorescent substrate
703102	Glutathione Peroxidase Assay Kit	Measure glutathione-dependent peroxidases in plasma, erythrocyte lysate, tissue homogenates, and cell lysates
703202	Glutathione Reductase Assay Kit	Measure glutathione reductase activity in plasma, erythrocyte lysate, tissue homogenates, and cell lysates
11527	Thioredoxin Activity Fluorescent Assay Kit	Determine human thioredoxin 1 activity utilizing the reaction between reduced thioredoxin and insulin disulfides
10007892	Thioredoxin Reductase Colorimetric Assay Kit	Measure the reduction of DTNB with NADPH as a readout of thioredoxin reductase activity
11529	Thioredoxin Reductase Fluorescent Activity Assay Kit	Measure the reduction of fluorescent glutathione *via* the combined reaction of the thioredoxin system as a readout of thioredoxin reductase activity
11526	Thioredoxin/Thioredoxin Reductase Mammalian Assay Kit	Measure the activity of the thioredoxin system by determining the net increase in free thiols formed from the reduction of insulin disulfides

Redox Antibodies

Item No.	Product Name	Immunogen	Host	Application(s)
11546	Glutaredoxin 1 (human) Polyclonal Antibody - biotinylated	Human Grx1	Goat	ELISA, IHC, WB
11545	Glutaredoxin 1 (human) Pure Polyclonal Antibody	Human Grx1	Goat	IHC, WB
11544	Glutaredoxin 2 (human) Polyclonal Antibody	Human Grx2 amino acids 41-164	Rabbit	WB
11537	Thioredoxin 1 (*E. coli*) Polyclonal Antiserum	*E. coli* Trx1	Sheep	Functional blocking
11539	Thioredoxin 1 (human) Monoclonal Antibody (Clone 2G11)	Human Trx1	Mouse	ELISA, IF, IP, WB
11542	Thioredoxin 1 (human) Polyclonal Antibody	Human Trx1 peptide 84-105	Rabbit	ELISA, IHC, WB

Redox Antibodies Continued

Item No.	Product Name	Immunogen	Host	Application(s)
11541	Thioredoxin 1 (human) Polyclonal Antibody - biotinylated	Human Trx1	Goat	ELISA, IHC, WB
11538	Thioredoxin 1 (human) Pure Polyclonal Antibody	Human Trx1	Goat	IHC, WB
11540	Thioredoxin 1 (mouse) Polyclonal Antibody	Mouse Trx1	Rabbit	WB
11543	Thioredoxin 1 Truncated (human) Monoclonal Antibody (Clone 7D11)	N-terminal 80 amino acids of human Trx1	Mouse	ELISA, IF, IP, WB

Redox Enzymes

Item No.	Product Name
11533	Glutaredoxin 1 (human recombinant)
11530	Glutaredoxin 1 from *E. coli*
11534	Glutaredoxin 1 from *E. coli* (mutant C14S)
11532	Glutaredoxin 2 (human recombinant)
11520	Thioredoxin 1 (human mutant C61S/C72S)
11518	Thioredoxin 1 (human recombinant)

Item No.	Product Name
11521	Thioredoxin 1 (mouse recombinant)
11517	Thioredoxin 1 from *E. coli* (oxidized form)
11522	Thioredoxin 1 Truncated (human recombinant)
14638	Thioredoxin Reductase 1 (rat recombinant)
11523	Thioredoxin Reductase from *E. coli*

IMCO

The IMCO Corporation of Sweden is a major manufacturer of products developed for research on thioredoxins, thioredoxin reductases, and glutaredoxins. Cayman is pleased to partner with IMCO in delivering these high-quality products to researchers in North America and around the world.

Bulk quantities and convenient pack sizes are available for many of our research reagents.

Request a quote for a volume discount at **www.caymanchem.com**

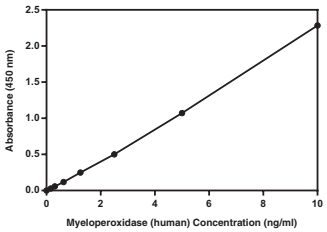
Myeloperoxidase

Myeloperoxidase (MPO) is one of the main weapons released by neutrophils and monocytes to battle bacteria and other invading pathogens. By measuring the presence, activity, or artifacts of this enzyme, researchers can better understand another facet of the complex (im)balance of redox signaling in their experimental models. Cayman provides researchers with assays that have been validated on activated neutrophils to ensure reproducible results. Each assay includes all necessary substrates, buffers, and positive controls to effectively measure the presence, activity, and inhibition of MPO. Antibodies are also available to visualize this enzyme in both human and mouse tissues by immunofluorescence.

Assay Kits

Myeloperoxidase (human) ELISA Kit 501410

- Detect human MPO in cell culture supernatants, plasma, and serum
- Assay 24 samples in triplicate or 36 samples in duplicate
- Measure human MPO levels down to 0.16 ng/ml
- Rapid assay; get results in under 4 hours

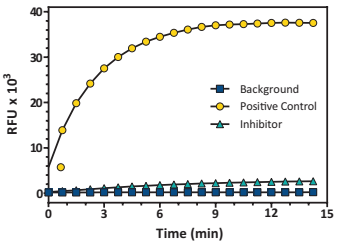


Assay Range = 0.16-10 ng/ml
Dynamic Range = 0.2-10 ng/ml
Sensitivity (defined as LLOQ) = 0.2 ng/ml
Lower Limit of Detection = 0.0583 ng/ml

The sensitivity and mid-point were derived from the standard curve shown above. The standard was diluted with ELISA Buffer.

Myeloperoxidase Peroxidation Fluorometric Assay Kit 700160

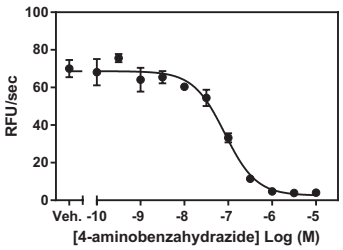
- Measure MPO peroxidase activity in cell lysates and purified preparations
- Assay 37 samples in duplicate
- Plate-based fluorometric measurement (ex 530-540 nm, em 585-595 nm)



Also Available: Myeloperoxidase Chlorination Fluorometric Assay Kit (Item No. 10006438)

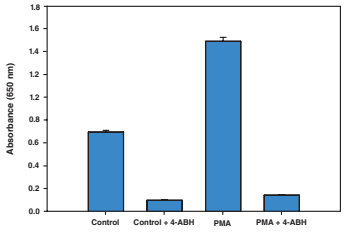
Myeloperoxidase Inhibitor Screening Assay Kit 700170

- Screen for inhibitors of MPO
- Includes reagents to measure both the chlorination and peroxidation activities
- Assay 45 samples in duplicate
- Plate-based fluorometric measurement
  (ex 480-495 nm, em 515-525 nm; chlorination assay)
  (ex 530-540 nm, em 585-595 nm; peroxidation assay)



Neutrophil Myeloperoxidase Activity Assay Kit 600620

- Assay the release of enzymatically-active MPO from activated phagocytes
- Utilizes TMB as a chromogenic substrate for MPO
- Includes a specific inhibitor of MPO function to verify specificity
- Includes reagents needed to isolate neutrophils from human whole blood



Monoclonal Antibodies

Item No.	Product Name	Host	Species Reactivity	Application(s)
15638	Myeloperoxidase Monoclonal Antibody (Clone 2C8)	Mouse	(+) Human MPO (+) Mouse MPO	IF
15639	Myeloperoxidase Monoclonal Antibody (Clone 4E9)	Mouse	(+) Human MPO (+) Mouse MPO	IF
15640	Myeloperoxidase Monoclonal Antibody (Clone 5F6)	Mouse	(+) Human MPO (+) Mouse MPO	IF

Biomarkers for Oxidative Damage/Activity

Drawing from more than 20 years of experience in the field of nitric oxide and oxidative injury, Cayman has developed a wide variety of reagents and assays to measure oxidation of macromolecules including lipids, proteins, and nucleic acids. Mass spectrometry standards, LC-MS mixtures, and an extensive array of highly sensitive assays are available to measure ROS or the damage produced by ROS as evidenced by accepted biomarkers.

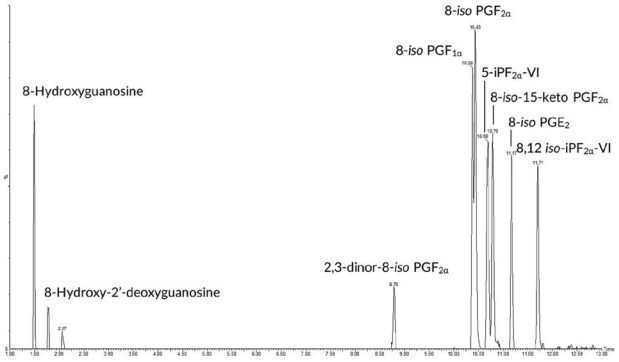
LC-MS Mixture

Oxidative Stress LC-MS Mixture 18701

1 ampule (1 µg/ml of each compound)

Contains:

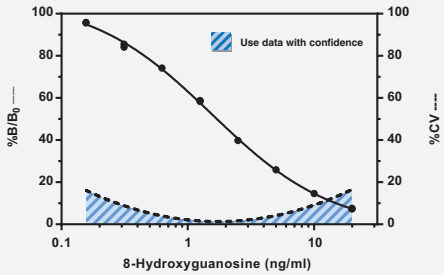
- 8-Hydroxyguanosine
- 8-Hydroxy-2'-deoxyguanosine
- 2,3-dinor-8-*iso* Prostaglandin $F_{2\alpha}$
- 8-*iso* Prostaglandin $F_{1\alpha}$
- 8-*iso* Prostaglandin $F_{2\alpha}$
- 5-$iPF_{2\alpha}$-VI
- 8-*iso*-15-keto Prostaglandin $F_{2\alpha}$
- 8-*iso* Prostaglandin E_2
- 8,12-*iso*-$iPF_{2\alpha}$-VI



Assay Kits

DNA/RNA Oxidative Damage (Clone 7E6.9) ELISA Kit 501130

- Measure DNA oxidative damage marker 8-hydroxy-2'-deoxyguanosine and RNA damage marker 8-hydroxyguanosine with equal selectivity and sensitivity
- Cross reactivity of clone 7E6.9 validated by mass spectrometry
- Assay 24 samples (urine or other matrices) in triplicate or 36 samples in duplicate
- Measure 8-hydroxyguanosine levels down to 0.45 ng/ml
- **Incubation:** 18 hours | **Development:** 90-120 minutes
  Read: Colorimetric at 405-420 nm



Assay Range = 0.15-20 ng/ml
Sensitivity (defined as 80% B/B_0) = 0.45 ng/ml
Mid-point (defined as 50% B/B_0) = 1.25-1.75 ng/ml

The sensitivity and mid-point were derived from the standard curve shown above. The standard was diluted with ELISA Buffer.

Additional Assay Kits

Item No.	Product Name	Summary
589320	DNA/RNA Oxidative Damage ELISA Kit	Measure major oxidative damage markers, 8-hydroxy-2'-deoxyguanosine, 8-hydroxyguanosine, and 8-hydroxyguanine in urine, cell culture medium, cell lysates, tissue samples, saliva, and plasma/serum samples
600050	Hydrogen Peroxide Cell-Based Assay Kit	A simple fluorometric assay for the quantification of extracellular H_2O_2
516351	8-Isoprostane ELISA Kit ***Also Available:*** 8-Isoprostane Express ELISA Kit (Item No. 516360)	Measure 8-isoprostane, a biomarker of oxidative stress and antioxidant deficiency
705002	Lipid Hydroperoxide (LPO) Assay Kit ***Also Available:*** Lipid Hydroperoxide (LPO) Assay Kit (96 well) (Item No. 705003)	Measure lipid hydroperoxides in tissues, cultured cells, plant materials, foods, and biological fluids

For a full comparison of the antibodies used in Item Nos. 501130 and 589320, download our scientific poster "Critical comparison of three 8-hydroxy-2'-deoxyguanosine monoclonal antibodies" at **www.caymanchem.com/Literature/scientificposters.**

Additional Assay Kits Continued

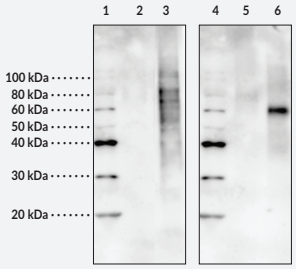
Item No.	Product Name	Summary
600160	Methionine Sulfoxide Immunoblotting Kit	Detect proteins containing methionine sulfoxide residues by Western blot
10005020	Protein Carbonyl Colorimetric Assay Kit	Measure oxidized proteins *via* the reaction between DNPH and protein carbonyls
10009055	TBARS Assay Kit ***Also Available:*** TBARS (TCA Method) Assay Kit (Item No. 700870)	Measure thiobarbituric acid reactive substances in plasma, serum, urine, tissue homogenates, and cell lysates
10010895	Xanthine Oxidase Fluorometric Assay Kit	Measure xanthine oxidase activity in plasma, serum, and tissue homogenates

Nitrotyrosine

Nitrotyrosine is formed by peroxynitrite-mediated nitration of protein tyrosine residues. Its presence on proteins can be used as a marker for peroxynitrite formation *in vivo*.

Nitrotyrosine IP Kit 601220

- Determine change in tyrosine nitration in treated *versus* non-treated samples
- Demonstrate target proteins are nitrated *in vitro*
- Identify and characterize nitrated proteins by Western blot and proteomic analysis



Lane 1: MW Markers
Lane 2: Unmodified Raji Cell Lysates
Lane 3: Nitrated Raji Cell Lysates
Lane 4: MW Markers
Lane 5: Unmodified BSA
Lane 6: Nitrated BSA

Western blot analysis of Nitrotyrosine IP Kit. Peroxynitrite-treated and control cell lysates as well as the supplied positive control were run on a 12% gel, transferred to nitrocellulose, and probed with the Anti-Nitrotyrosine Polyclonal Antibody. The data demonstrate that both the Affinity Sorbent and the Polyclonal Antibody recognize only nitrotyrosine containing proteins.

Nitrotyrosine Antibodies

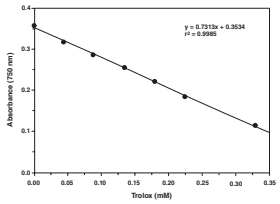
Item No.	Product Name	Host	Species Reactivity	Application(s)
189542	Nitrotyrosine Monoclonal Antibody	Mouse	(+) Species-independent detection of nitrotyrosine	ELISA, WB
10006966	Nitrotyrosine Monoclonal Antibody - Biotinylated	Mouse	(+) Species-independent detection of nitrotyrosine	ELISA, IHC, IP, WB
10189540	Nitrotyrosine Polyclonal Antibody	Rabbit	(+) Species-independent detection of nitrotyrosine	WB
10006778	Nitrotyrosine (Peptide) Polyclonal Antibody	Rabbit	(+) Species-independent detection of nitrotyrosine, less than 10% reactivity with chlorotyrosine (-) Synthetic peptide containing unmodified tyrosine	WB

Antioxidant Detection/Activity

Cayman manufactures a variety of assay kits to evaluate many of the primary antioxidants used as protection against ROS. Each kit conveniently packages the reagents needed to detect the activity of key enzymes or small molecules involved in the antioxidant system. These assays have been optimized for sensitivity and reproducibility by our in-house scientists for reliable quantification of antioxidant activity.

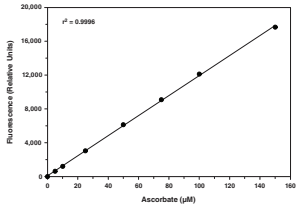
Antioxidant Assay Kit 709001

- Measure the total antioxidant capacity of plasma, serum, urine, saliva, or cell lysates
- Assay 41 samples in duplicate
- Measure antioxidant capacity in Trolox equivalents down to 44 µM
- Plate-based colorimetric measurement (750 or 405 nm)



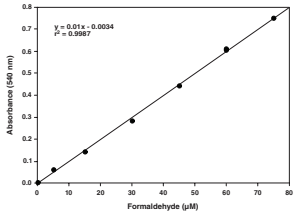
Ascorbate Assay Kit 700420

- Quantify ascorbate from plasma, serum, urine, and fruit juices
- Assay 40 samples in duplicate
- **Assay Range:** 5-150 µM
- Plate-based fluorometric measurement (ex 340-350 nm, em 420-430 nm)



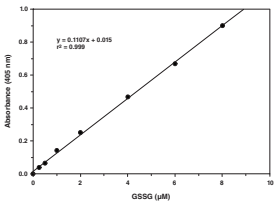
Catalase Assay Kit 707002

- Measure catalase activity in plasma, serum, erythrocyte lysates, tissue homogenates, and cell lysates
- Assay 40 samples in duplicate
- Measure catalase activity down to 2 U/ml
- Plate-based colorimetric measurement (540 nm)



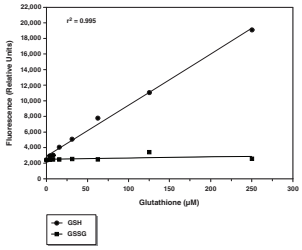
Glutathione Assay Kit 703002

- Measure total oxidized (GSSG), and/or reduced (GSH) glutathione in cell lysates, tissue homogenates, plasma and erythrocyte lysates, and serum
- Assay 40 samples in duplicate
- **Assay Range:** 0.25-8 µM (GSSG) or 0.5-16 µM (GSH)
- Plate-based colorimetric measurement (405-414 nm)



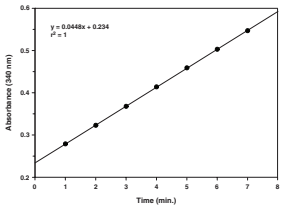
Glutathione Cell-Based Detection Kit (Blue Fluorescence) 600360

- Utilizes MCB, a highly fluorescent GSH probe
- Includes a standard curve for accurate quantification of GSH from cell lysates
- Rapid assay; get results in 2 hours



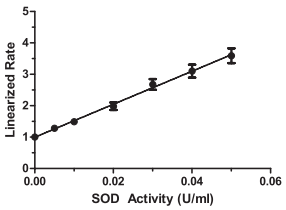
Glutathione S-Transferase Assay Kit 703302

- Measure GST activity in plasma, tissue homogenates, and cell lysates
- Assay 22 samples in duplicate
- Measure GST activity down to 24 U/ml
- Plate-based colorimetric measurement (340 nm)



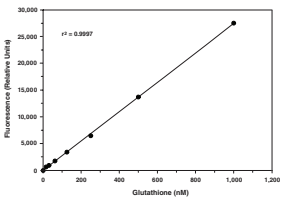
Superoxide Dismutase Assay Kit 706002

- Measure copper/zinc, manganese, and iron SOD in tissues, plasma, serum, and cell lysates
- Assay 41 samples in duplicate
- Measure SOD activity down to 0.005 U/ml



Thiol Detection Assay Kit 700340

- Measure free thiol content in plasma, serum, urine, cell lysates, and tissue homogenates
- Assay 40 samples in duplicate
- **Assay Range:** 15 nm-1 µM
- Plate-based fluorometric measurement (ex 380-390 nm, em 510-520 nm)
- Includes both a glutathione and cysteine standard



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