Product Manual

OxiSelect™ Protein Radical ELISA Kit

Catalog Number

STA-810 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Reactive nitrogen species (RNS) such as peroxynitrite ($ONOO^-$) and reactive oxygen species (ROS) such as peroxyl radicals (ROO·), hydroxyl radicals (OH·), or superoxide radical anions have been implicated in protein radical formation through the removal of an electron or hydrogen atom. These protein radicals can lead to internal crosslinking of the polypeptide chain, cause protein backbone cleavage, and form protein peroxides as well as peroxyl radicals. The formation of protein radicals has been linked to various disorders including amyotrophic lateral sclerosis, aging, Huntington's Disease, and Alzheimer's disease.

Radical formation results in an unstable molecule that contains an atom with one unpaired electron in its outer orbital, giving the atom unique paramagnetic properties. These properties allow for the use of the electron spin resonance (ESR) technique to detect various forms of biological radicals. Since radicals are very unstable, an ESR spin trapping technique was developed to increase detection sensitivity. ESR spin trapping involves a reaction between the reactive protein radical and a diamagnetic molecule (such as 3,4-dihydro-2,2-dimethyl-2H-pyrioole 1-oxide, or DMPO) also known as a spin trap. This reaction results in a more stable DMPO radical adduct that can be detected by ESR. More recently, an "immuno-spin trapping" technique has been used to detect radical formation (Figure 1). It has been demonstrated that the aforementioned DMPO radical adduct decays into a DMPO nitrone adduct due to oxidation. Antibodies raised against the stable DMPO nitrone adduct recognize DMPO bound to protein, and can be used to demonstrate protein radical formation in an ELISA or western blot format.

The OxiSelect™ Protein Radical ELISA Kit is an immunoassay developed for rapid detection and quantitation of protein radicals. The relative amount of protein radicals within a protein sample is determined by comparing its absorbance with that of a known DMPO Nitrone Adduct Human Serum Albumin (HSA) standard curve. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown protein samples.

Assay Principle

DMPO Nitrone Adduct-HSA standard or DMPO adduct protein samples are adsorbed onto a 96-well plate for 2 hrs at 37ºC or 4ºC overnight. The DMPO protein adducts present in the sample are probed with an anti-DMPO Nitrone Adduct antibody, followed by an HRP conjugated secondary antibody. The protein radical content in the unknown sample is determined by comparing with a standard curve that is prepared from predetermined DMPO Nitrone Adduct-HSA standards.

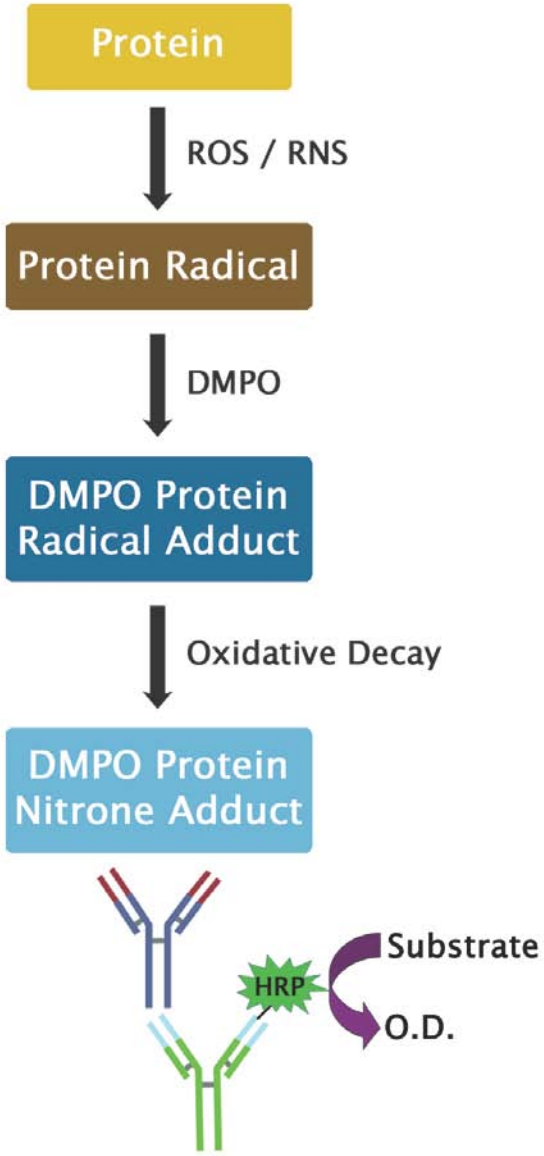

Figure 1. Immuno-spin trapping technique for the detection of protein radicals.

Related Products

1. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
2. STA-318: OxiSelect™ AOPP Assay Kit
3. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
4. STA-330: OxiSelect™ TBARS Assay Kit (MDA Quantitation)
5. STA-342: OxiSelect™ Intracellular ROS Assay Kit (Green Fluorescence)
6. STA-343: OxiSelect™ Hydrogen Peroxide Assay Kit

7. STA-347: OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence)
8. STA-816: OxiSelect™ N-epsilon-(Carboxymethyl) Lysine (CML) Competitive ELISA Kit
9. STA-817: OxiSelect™ Advanced Glycation End Products (AGE) Competitive ELISA Kit
10. STA-832: OxiSelect™ MDA Adduct Competitive ELISA Kit
11. STA-838: OxiSelect™ HNE Adduct Competitive ELISA Kit

Kit Components

1. 96-well Protein Binding Plate (Part No. 231001): One strip well 96-well plate.
2. Anti-DMPO Nitrone Adduct Antibody (1000X) (Part No. 281001): One 10 µL vial of anti-DMPO Rabbit IgG.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 231009): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.
8. DMPO Nitrone Adduct HSA standard (Part No. 281002): One 10 µL vial of 0.4 mg/mL DMPO Nitrone Adduct HSA in PBS.
9. Reduced BSA Standard (Part No. 233202): One 500 µL vial of 1 mg/mL reduced BSA in PBS.

Materials Not Supplied

1. DMPO
2. H_2O_2
3. $CuSO_4$
4. Protein samples such as purified protein, plasma, serum, cell lysate, or tissue homogenate
5. 1X PBS
6. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
7. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
8. Multichannel micropipette reservoir
9. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the DMPO Nitrone Adduct HSA and Reduced BSA Standard at -20ºC to avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-DMPO Nitrone Adduct Antibody and Secondary Antibody: Immediately before use, dilute the Anti-DMPO Nitrone Adduct antibody 1:1000 and Secondary Antibody 1:1000 with 1X Assay Diluent. Do not store diluted solutions.

Preparation of DMPO Nitrone Adduct HSA Standards

1. Freshly prepare 10 µg/mL of Reduced BSA by diluting the 1 mg/mL BSA standard 1:100 into 1X PBS.
2. Freshly prepare 100 ng/mL of DMPO Nitrone Adduct HSA by diluting the 0.4 mg/mL DMPO Nitrone Adduct HSA standard 1:4000 into 10 µg/mL of Reduced BSA. Example: Add 2 µL to 8 mL of 10 µg/mL Reduced BSA.
3. Prepare a series of DMPO Nitrone Adduct HSA standards according to Table 1.

Standard Tubes	100 ng/mL DMPO Nitrone Adduct HSA (µL)	10 µg/mL Reduced BSA (µL)	DMPO Nitrone Adduct HSA (ng/mL)
1	1000	0	100
2	500 of Tube #1	500	50
3	500 of Tube #2	500	25
4	500 of Tube #3	500	12.5
5	500 of Tube #4	500	6.25
6	500 of Tube #5	500	3.125
7	500 of Tube #6	500	1.5625
8	0	500	0

Table 1. Preparation of DMPO Nitrone Adduct HSA Standard Curve

Preparation of Samples

1. Treat samples (cells, lysates, plasma, or serum) with 100 mM DMPO, 4.4 mM H2O2 and 50 µM CuSO4 for 18-24 hours at 37ºC. If treating intact cells, prepare whole cell lysates following treatment.

   Notes for cell and tissue lysates:
   - *Lysates should not be prepared in lysis buffer containing Triton X-100, NP-40, or Igepal CA-630 because these detergents interfere with protein coating of the plate unless the detergent concentration in the 1 µg/mL protein samples is no more than 0.001%. We recommend lysis by homogenization, sonication, or freeze-thaw.*
   - *Avoid using Tris buffer when treating cells or lysates with DMPO since Tris inhibits Nitrone adduct formation. We recommend using a 100 mM sodium phosphate dibasic pH 7.4 solution.*

2. Perform a protein assay such as Bradford or BCA on all samples to determine the protein concentration.

3. Dilute each protein sample to 10 µg/mL prior to use in the assay.

Assay Protocol

1. Prepare unknown samples according to the Preparation of Samples section above. Each 10 µg/mL protein sample and DMPO Nitrone Adduct HSA Standard should be assayed in duplicate or triplicate.
2. Add 100 µL of 10 µg/mL protein samples or DMPO Nitrone Adduct HSA standards to the 96-well Protein Binding Plate. Incubate at 37ºC for at least 2 hours or 4ºC overnight.
3. Wash wells 3 times with 250 µL 1X PBS per well. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess wash solution.
4. Add 200 µL of Assay Diluent per well and incubate for 1 hour at room temperature.
5. Wash 3 times with 250 µL of 1X Wash Buffer with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
6. Add 100 µL of the diluted anti-DMPO Nitrone Adduct Antibody to all wells and incubate for 1 hour at room temperature on an orbital shaker. Wash the strip wells 3 times according to step 5 above.
7. Add 100 µL of the diluted Secondary Antibody, HRP Conjugate to all wells and incubate for 1 hour at room temperature on an orbital shaker. Wash the strip wells 3 times according to step 5 above.
8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
9. Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time).
10. Read absorbance of each well on a plate reader using 450 nm as the primary wave length. Using the fully reduced BSA standard as absorbance blank.

Example of Results

The following figures demonstrate typical Protein Radical ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

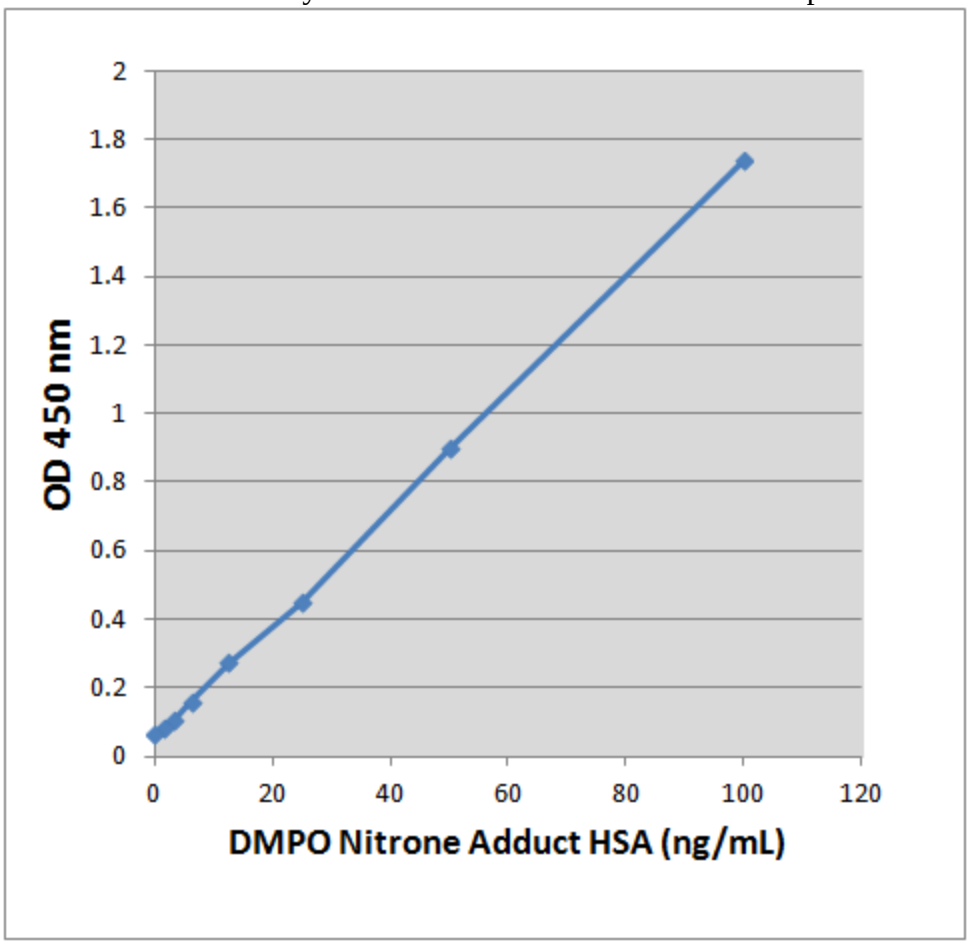

Figure 2. OxiSelect™ Protein Radical ELISA Kit Standard Curve.

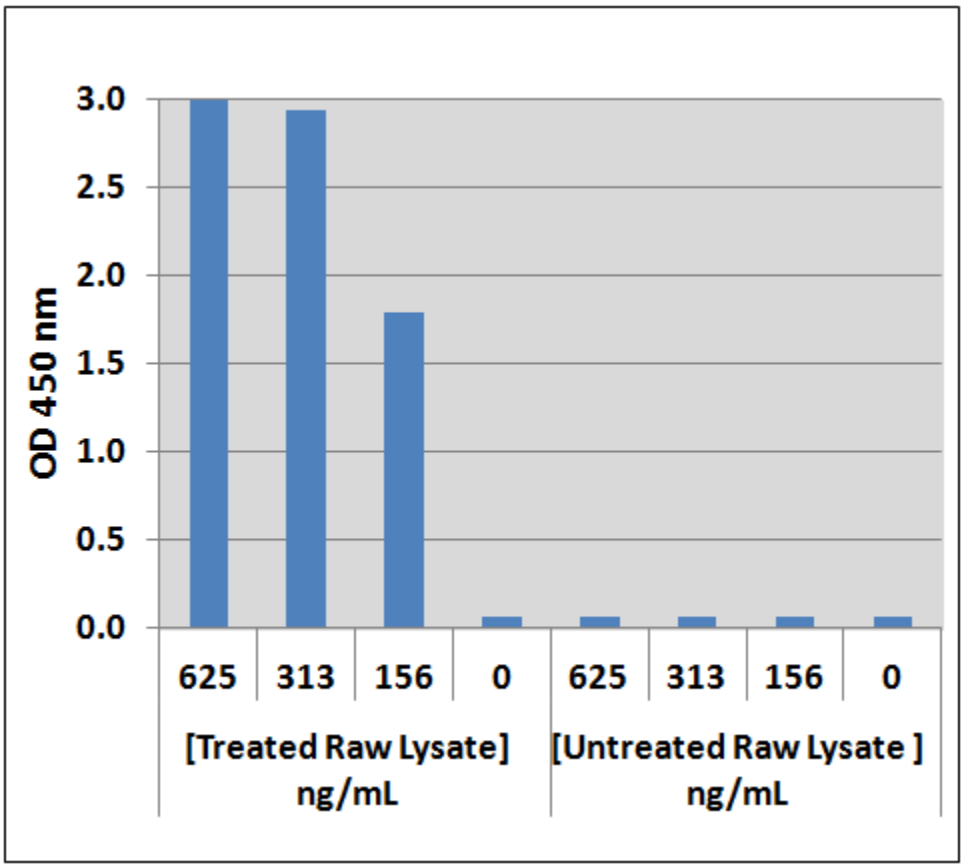

Figure 3. Detection of Protein Radicals in Raw 264.7 Cell Lysate. Raw 264.7 macrophages were trypsinized, washed, subjected to three freeze thaw cycles, and centrifuged. The resulting lysate was treated overnight at 37ºC with 100 mM DMPO in the presence (left, "Treated") or absence (right, "Untreated") of 4.4 mM H2O2 and 50 µM CuSO4. Lysates were tested using the OxiSelect™ Protein Radical ELISA Kit.

References

1. Lardinois OM., Detweiler CD., Tomer KB., Mason RP., and Deterding LJ. (2008) *Free Radic Biol Med*. **44**: 893-906.
2. Dalle-Donne I., Scaloni A., Giustarini D., Cavarra E., Tell G.,m Lungarella G., Colombo R., Rossi R., and Milzani A., (2005) *Mass Spec Rev*., **24**:55-99.
3. Davies MJ., Fu S., Wang H., and Dean RT., (1999*) Free Radic Biol Med*., **27**:1151-1163.
4. Chen C-A., Lin C-H, Druhan LJ, Wang T-Y, Chen Y-R., and Zweier JL., (2011) *J. Biol Chem* **286**: 29098-29107.

Warranty

In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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