Product Manual

OxiSelect™ Protein Carbonyl Spectrophotometric Assay

Catalog Number

STA-315 **40 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Protein oxidation is defined as the covalent modification of a protein induced either directly by reactive oxygen species (ROS) or indirectly by reaction with secondary by-products of oxidative stress. Oxidative modification of proteins can be induced in vitro by a wide array of pro-oxidant agents and occurs in vivo during aging and in certain disease conditions.

There are numerous types of protein oxidative modification. The most common products of protein oxidation in biological samples are the protein carbonyl derivatives of Pro, Arg, Lys, and Thr. These derivatives are chemically stable and serve as markers of oxidative stress for most types of ROS. Many of the current assays involve derivitization of the carbonyl group with dinitrophenylhydrazine (DNPH), followed by an anti-DNP antibody detection. When DNPH reacts with protein carbonyls, the amount of protein-hydrozone produced can also be measured spectrophotometrically at 375 nm.

Cell Biolabs' Protein Carbonyl Spectrophotometric Assay provides a convenient system to measure the protein carbonyl content in plasma, serum, cell lysates and purified proteins. Oxidized BSA is included as assay positive controls. Each kit provides sufficient reagents to perform up to 40 assays including unknown protein samples, assay blank and oxidized BSA controls.

Assay Principle

Protein carbonyls in BSA standards or protein samples (1-10 mg/mL) are derivatized with DNPH first. Proteins are then TCA precipitated and free DNPH is removed by washing the protein pellet. After dissolving the protein pellet in GuHCl, the absorbance of protein-hydrozone is measured at 375 nm, and the protein carbonyl is calculated.

Related Products

1. STA-305: OxiSelect™ Nitrotyrosine ELISA Kit
2. STA-308: OxiSelect™ Protein Carbonyl Immunoblot Kit
3. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
4. STA-318: OxiSelect™ AOPP Assay Kit
5. STA-332: OxiSelect™ MDA ELISA Kit
6. STA-330: OxiSelect™ TBARS Assay Kit (MDA Quantitation)
7. STA-334: OxiSelect™ HNE Adduct ELISA Kit
8. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG)
9. STA-325: OxiSelect™ Oxidative RNA Damage ELISA Kit (8-OHG)

Kit Components

1. DNPH (Part No. 231004): One 200 mg amber vial.

2. 2X DNPH Diluent (Part No. 231501): One 50 mL bottle.
3. 5X TCA Solution (Part No. 231502): One 50 mL bottle.
4. Ethyl Acetate (Part No. 231503): One 120 mL amber glass bottle.
5. Protein Solubilization Solution (Part No. 231504): One 15 mL bottle.
6. Oxidized BSA Positive Control (Part No. 231505): One 1 mL vial of 2 mg/mL BSA in PBS.

Materials Not Supplied

1. 1 to 10 mg/mL Protein samples such as plasma, serum, cell lysate, purified protein.
2. Protein assay such as Pierce BCA or Bradford assay.
3. Ethanol
4. Streptomycin sulfate
5. Spectrophotometer or plate reader capable of measuring absorbance 375 nm.

Storage

Upon receipt, aliquot and store the Oxidized BSA Standard at -20 ºC to avoid multiple freeze/thaw cycles. Store all other components at 4 ºC until their expiration dates.

Preparation of Reagents

- 1X TCA Solution: Dilute the 5X TCA Solution to 1X with deionized water. Stir to homogeneity.
- DNPH Solution: Dilute 2X DNPH Diluent to 1X with deionized water. Weigh out 1-5 mg of DNPH and dissolve in 1X DNPH Diluent to make a 2 mg/mL DNPH solution. This solution is stable for one week when stored in the dark at 4 ^{0}C.
- Ethanol/Ethyl Acetate Wash Solution: Immediately before use, mix equal volumes of ethanol and ethyl acetate in a glass container. Use immediately. Do not store wash solution.

Preparation of Samples

1. Prepare cell or tissue lysate samples at 1 to 10 mg/mL.
2. Perform a Protein Assay.

   Note: For protein samples lower than 1 mg/mL, concentrate by centricon filtration or by mixing 0.8 vol of protein sample and 0.2 vol of 5X TCA solution, incubating 10 minutes on ice and centrifuging at 10,000 g. Dissolve the protein pellet in a small volume of 1X PBS.
3. (Optional) High nucleic acid can erroneously contribute to higher estimation of carbonyls. To remove nuclei acid, add streptomycin sulfate or PEI to a final concentration of 1% and 0.5%

respectively, incubate 30 minutes at room temperature and remove the nuclei acid precipitates by centrifuging at 6000 g for 10 minutes at 4 ^{0}C.

Assay Protocol

1. Add 1.0 mL of DNPH Solution to 250 µL of protein sample (1-10 mg/mL), incubate for 45 minutes at room temperature in the dark with occasional mixing. A blank protein sample that reacts with 1X DNPH Diluent only (no DNPH) is required for each sample.
2. Add 1.25 mL of 1X TCA Solution, keep on ice for 10 minutes. Centrifuge for 10 minutes at 3,000 g to 10,000 g at 4 ^{0}C.
3. Discard the supernatant, and wash the pellet 5 times with 1 mL of ethanol/ethyl acetate (1:1, v/v) thoroughly.
4. After the final wash, resuspend the protein pellet in 250 µL of Protein Solubilization Solution. Vortex thoroughly and incubate 10 minutes at 37 ^{0}C. Centrifuge for 10 minutes at 10,000 g to remove any debris. Repeat the centrifugation step if needed.
5. Determine the protein concentration of the solubilized protein sample by BCA or Bradford Protein Assay. The solubilized protein sample must be diluted at least 2 fold with dH2O before BCA or Bradford Protein Assay.
6. Transfer supernatant to an minicuvette for spectrophotometer or a 96-well plate for a plate reader and measure the absorbance at 375 nm. The absorbance is read against the blank of each sample.

Calculation of Results

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

Calculations:

The extinction coefficient (ε) for dinitrophenylhydrazine at 375 nm is 22,000 M^{-1} cm^{-1}. When the absorbance of the sample is read at 375 nm against its blank, the carbonyl content is calculated:

Protein Carbonyl (M) = A375 nm / 22,000 M^{-1} (when 1 cm-width cuvette is used)
Protein Carbonyl (nmol/mL) = A375 nm x 45.45 (nmol/mL)
Protein Carbonyl (nmol/mg) = Protein Carbonyl (nmol/mL) / Protein Concentration (mg/mL)

Calculation Example:

2 mg/mL of BSA solution is derivatized with DNPH first. After TCA precipitation and wash, the protein pellet is dissolved in Protein Solubilization Solution and the protein concentration of the solubilized protein is 1.5 mg/mL determined by BCA assay. OD 375 nm of protein-DNP solution is 0.150.

Protein Carbonyl (nmol/mL) = A375 nm x 45.45 (nmol/mL) = 0.15 x 45.45 = 6.8 nmol/mL
Protein Carbonyl (nmol/mg) = Protein Carbonyl (nmol/mL) / Protein Concentration (mg/mL)
= 6.8 nmol/mL/1.5 mg/mL
= 4.5 nmol/mg

References

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5. Reznick, AZ., and Packer, L. (1994) Methods Enzymol. 233: 263-357.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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