



Protein Carbonyl ELISA Kit

Cat. No.

STA-310: One kit, 96 tests

STA-310-5: Five kits, 5 x 96 tests

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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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 immunoblotting with an anti-DNP antibody. The Protein Carbonyl ELISA was first developed by Buss and co-workers, the protein samples (≥ 4 mg/mL) react with DNPH and then adsorb to wells of an ELISA plate before probe with anti-DNPH antibody. In their method, protein samples containing low amounts of protein must be concentrated to at least 4 mg/mL by TCA precipitation. However, TCA precipitation results a 20% loss of the total carbonyl values, and loss of protein during precipitation is also expected. In Cell Biolabs' Protein Carbonyl ELISA Kit, protein samples are first allowed to adsorb to wells of a 96-well plate and then react with DNPH. There is no need to concentrate protein in experimental and clinical samples with low amounts of protein (< 4 mg/mL) and the kit requires protein sample as little as 10 μ g/mL.

Cell Biolabs' Protein Carbonyl ELISA Kit is an enzyme immunoassay developed for rapid detection and quantitation of protein carbonyls. The quantity of protein carbonyls in protein sample is determined by comparing its absorbance with that of a known reduced/oxidized BSA standard curve. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown protein samples.

Cell Biolabs' Protein Carbonyl ELISA Kit is intended for research use only, and not for diagnostic applications.

Related Products

1. STA-308: Protein Carbonyl Immunoblot Kit
2. STA-309: Carbonyl-BSA
3. STA-315: Protein Carbonyl Spectrophotometric Assay
4. STA-303: Nitrotyrosine Immunoblot Kit
5. STA-304: Nitrotyrosine-BSA

Test Principle

BSA standards or protein samples (10 μ g/mL) are adsorbed onto a 96-well plate for 2 hrs at 37 $^{\circ}$ C. The protein carbonyls present in the sample or standard are derivatized to DNP hydrazone and probed with an anti-DNP antibody, followed by an HRP conjugated secondary antibody. The protein carbonyl content in unknown sample is determined by comparing with a standard curve that is prepared from predetermined reduced and oxidized BSA standards.

Kit Components

1. 96-well Protein Binding Plate (Part No. 231001): One strip well 96-well plate.
2. Anti-DNP Antibody (1000X) (Part No. 231002): One 20 μ L vial of anti-DNP Rabbit IgG.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 231003): One 20 μ L vial.
4. DNPH (Part No. 231004): One 200 mg amber vial.
5. DNPH Diluent (Part No. 231005): One 50 mL bottle.
6. Blocking Reagent (Part No. 231006): One 20 g bottle.
7. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
8. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
9. Stop Solution (Part. No. 310808): One 12 mL bottle.
10. Reduced BSA Standard (Part No. 231007): One 200 μ L vial of 1 mg/mL fully reduced BSA in PBS.
11. Oxidized BSA Standard (Part No. 231008): One 200 μ L vial of 1 mg/mL oxidized BSA in PBS at 7.5 nmol protein carbonyl/mg proteins. The protein carbonyl is predetermined by a spectrophotometric method as described by Reznick and Parker (See Ref. 5).

Materials Not Supplied

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

Storage

Upon receiving, aliquot and store the reduced or oxidized BSA standard at -20°C , avoiding multiple freeze/thaws. Store all other kit components at 4°C until their expiration dates.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Blocking Solution: Weigh out 5 g of Blocking Reagent, dissolve in 100 mL of 1X PBS, and store at 4°C for up to one week.
- DNPH Working Solution: Weigh out 1-5 mg of DNPH, and dissolved in DNPH diluent to 1 mg/mL. This DNPH stock solution is stable for one week when stored in the dark at 4°C . Based on the number of tests, FRESHLY prepare appropriate amount of DNPH Working Solution by diluting the 1 mg/mL stock DNPH

solution to 0.04 mg/mL in DNPH diluent. Example: for 20 assays, transfer 100 μ L of 1 mg/mL DNPH stock solution to a tube containing 2.4 mL of DNPH Diluent, mix well and use it IMMEDIATELY.

- Anti-DNP Antibody and Secondary Antibody: Immediately before use dilute the Anti-DNPH antibody 1:1000 and Secondary Antibody 1:1000 with 1X Blocking Solution. Do not store diluted solutions.

Preparation of Protein Carbonyl BSA Standards

1. Freshly Prepare 10 μ g/mL of reduced or oxidized BSA by diluting the 1 mg/mL BSA standards in 1X PBS.
Example: Add 20 μ L to 1.98 mL of 1X PBS.
2. Prepare a series of carbonyl BSA standards according to Table 1.

Standard Tubes	Oxidized BSA (μ L)	Reduced BSA (μ L)	Protein Carbonyl (nmol/mg)
1	400	0	7.5
2	320	80	6.0
3	240	160	4.5
4	160	240	3.0
5	80	320	1.5
6	40	360	0.75
7	20	380	0.375
8	0	400	0

Table 1. Preparation of Protein Carbonyl BSA Standard Curve

Assay Instructions

1. Dilute unknown protein sample to 10 μ g/mL in 1X PBS. Each protein sample and BSA Standard should be assayed in duplicate or triplicate.

Note: High nucleic acid in cell or tissue lysate 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 $^{\circ}$ C.

2. Add 100 μ L of 10 μ g/mL protein samples, including reduced/oxidized BSA standards, to the 96-well Protein Binding Plate. Incubate at 37 $^{\circ}$ C for at least 2 hours or 4 $^{\circ}$ C overnight.
3. Wash wells 3 times with 250 μ L 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 100 μ L of the DNPH Working Solution and incubate for 45 minutes at room temperature in the dark.
5. Wash 5 times with 250 μ L of 1X PBS/Ethanol (1:1, v/v) 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 wash solution. Wash 2 times with 250 μ L of 1X PBS.

6. Add 200 μ L of Blocking Solution per well and incubate for 1-2 hours at room temperature on an orbital shaker.
7. Wash 3 times with 250 μ L 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.
8. Add 100 μ L of the diluted anti-DNP 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 7 above.
9. Add 100 μ L of the diluted HRP conjugated secondary antibody to all wells and incubate for 1 hour at room temperature on an orbital shaker. Wash the strip wells 5 times according to step 7 above.
10. Warm Substrate Solution to room temperature. Add 100 μ L of Substrate Solution to each well. Incubate at room temperature for 5-20 minutes on an orbital shaker.
11. Stop the enzyme reaction by adding 100 μ L of Stop Solution to each well. Results should be read immediately (color will fade over time).
12. 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.

Calculation and Example of Results

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

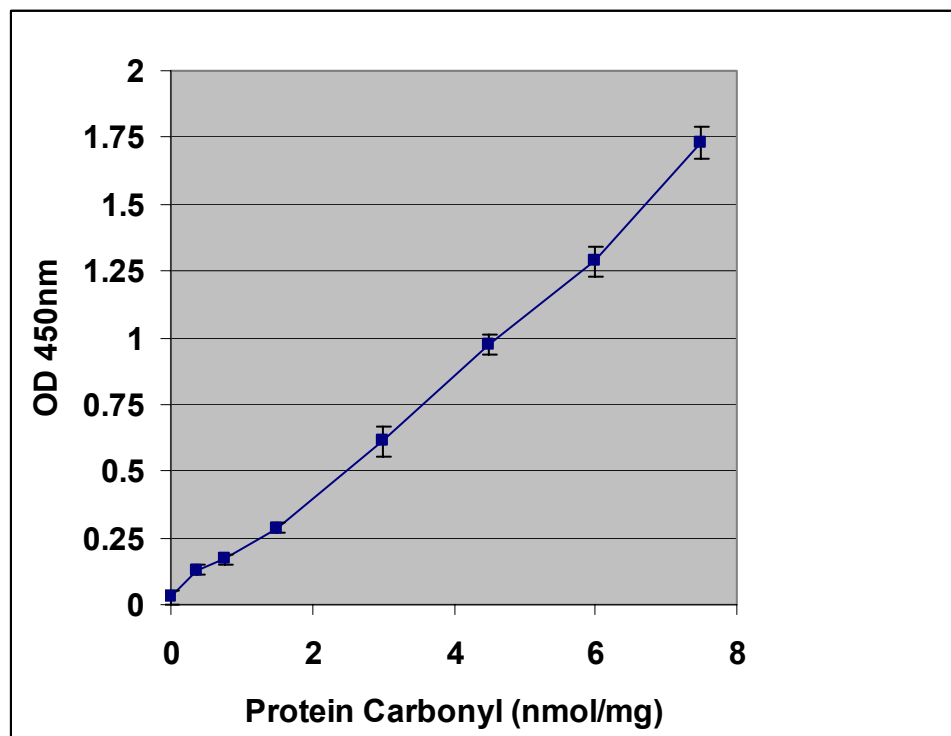


Figure 1: Protein Carbonyl ELISA Standard Curve.

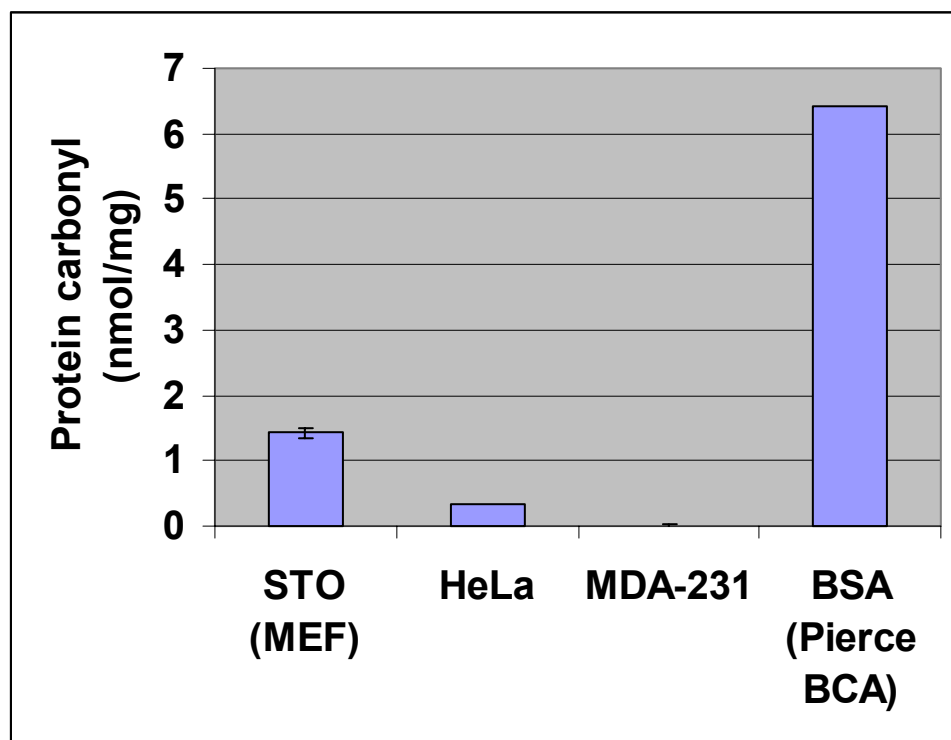


Figure 2: Amount of Protein Carbonyls for Cell Lysate and Pierce BCA BSA Standard. STO (MEF), HeLa and MDA-231 cells were lysed in 25mM HEPES, pH 7.5, 150 mM NaCl, 1% NP-40, 10 mM MgCl₂, 1

mM EDTA, 2% Glycerol. Cell Lysates and BSA Standard from Pierce BCA Protein Assay were diluted to 10 µg/mL with 1X PBS and coated onto a 96-well Protein Binding Plate. The protein carbonyl levels were determined as described in the assay instructions.

References:

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3. Talent, JM., Kong, Y., and Gracy, RW. (1998). *Anal. Biochem.* 31–38.
4. Buss, H, Chan, TP, Sluis, KB, Domigan, NM, and Winterbourn, CC. (1985) *Free Radic Biol Med.* 361-6.
5. Reznick, AZ., and Packer, L. (1994) *Methods Enzymol.* 233: 263-357.

Warranty

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