Datasheet for ABIN2344951

Protein Carbonyl ELISA Kit

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Overview

Quantity:	96 tests
Target:	Protein Carbonyl (PC)
Reactivity:	Others
Method Type:	Competition ELISA
Application:	ELISA

Product Details

Brand:	OxiSelect™
Sample Type:	Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Sensitivity:	10 µg/mL
Characteristics:	OxiSelect™ 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 µg/mL. The OxiSelect™ 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.
Components:	1. 96-well Protein Binding Plate : One strip well 96-well plate.

Product Details

	2. Anti-DNP Antibody (1000X) : One 20 µL vial of anti-DNP Rabbit IgG. 3. Secondary Antibody, HRP Conjugate (1000X) : One 20 µL vial. 4. DNPH : One 200 mg amber vial. 5. 2X DNPH Diluent : One 15 mL bottle. 6. Blocking Reagent : One 20 g bottle. 7. 10X Wash Buffer : One 100 mL bottle. 8. Substrate Solution : One 12 mL amber bottle. 9. Stop Solution (Part. No. 310808): One 12 mL bottle. 10. Reduced BSA Standard : One 200 µL vial of 1 mg/mL fully reduced BSA in PBS. 11. Oxidized BSA Standard : One 200 µL 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).
Material not included:	1. Protein samples such as purified protein, plasma, serum, cell lysate, or tissue homogenate 2. 1X PBS 3. Ethanol 4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips 5. 50 µL to 300 µL 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)

Target Details

Target:	Protein Carbonyl (PC)
Alternative Name:	Protein Carbonyl (PC Products)
Background:	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 derivatization of the carbonyl group with dinitrophenylhydrazine (DNPH), followed by immunobloting 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.

Target Details

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Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Detect as little as 10 µg/mL in a standard microplate reader • No concentration or precipitation steps that contribute to sample loss • Suitable for plasma, serum, cell lysates or purified proteins
Plate:	Uncoated
Protocol:	BSA standards or protein samples (10 µg/mL) are adsorbed onto a 96-well plate for 2 hrs at 37°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.
Reagent Preparation:	• 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity. 3 • 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 dissolve 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 µL 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 µL to 1.98 mL of 1X PBS. 2. Prepare a series of carbonyl BSA standards by mixing the oxidized BSA and reduced BSA in the proper ratios according to Table 1. 10 µ g/mL Oxidized 10 µg/mL Reduced [Protein Carbonyl] Standard Tubes BSA (µL) BSA (µL) (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
Sample Preparation:	1. Perform a protein assay such as Bradford or BCA on all samples to determine the protein concentration. 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 10 µg/mL protein samples is no more than 0.001 % . We recommend lysis by homogenization or sonication. • A

high concentration of nucleic acid in cell or tissue lysates can erroneously contribute to higher estimation of carbonyl content. To remove nucleic acid, we recommend one of the following procedures: 4

1. Pretreat lysate with nuclease, followed by ammonium sulfate precipitation of high percentage saturation.
2. 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 °C.
3. Dilute each protein sample to 10 µg/mL in 1X PBS prior to use in the assay. Note: Samples with high concentrations of protein carbonyl content may be further diluted 5-10 fold in 10 µg/mL Reduced BSA. A titration may be performed to ensure the samples fall in the range of the standard curve.

Assay Procedure:

1. Prepare unknown samples according to the Preparation of Samples section above. Each 10 µg/mL protein sample and BSA Standard should be assayed in duplicate or triplicate.
2. Add 100 µL of 10 µg/mL protein samples, including reduced/oxidized BSA standards, to the Protein Binding Plate. Incubate at 37 °C for at least 2 hours or 4 °C 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 100 µL of the DNPH Working Solution and incubate for 45 minutes at room temperature in the dark.
5. Wash wells with 250 µL of 1X PBS/Ethanol (1:1, v/v) with incubation on an orbital shaker for 5 minutes. Repeat washing a total of 5 times, aspirating between each. 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 µL of 1X PBS.
6. Add 200 µL of Blocking Solution per well and incubate for 1-2 hours at room temperature on an orbital shaker.
7. 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.
8. Add 100 µL 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 µL 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. 5
10. 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.
11. Stop the enzyme reaction by adding 100 µL 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.

Application Details

Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid multiple freeze/thaw cycles.
Storage:	4 °C/-20 °C
Storage Comment:	Upon receipt, aliquot and store both the Reduced and Oxidized BSA Standards at -20°C to avoid multiple freeze/thaw cycles. Store all other components at 4°C.

Publications

Product cited in:

Kim, Toyono, Berlinicke, Zack, Jurkunas, Usui, Jun: "Screening and Characterization of Drugs That Protect Corneal Endothelial Cells Against Unfolded Protein Response and Oxidative Stress. " in: **Investigative ophthalmology & visual science**, Vol. 58, Issue 2, pp. 892-900, (2017) (PubMed).

Wang, Yi, Tan, Zhang, Xu, Chen, Qin, Yu, Guan, Zhang: "Apigenin attenuates streptozotocin-induced pancreatic β cell damage by its protective effects on cellular antioxidant defense." in: **In vitro cellular & developmental biology. Animal**, (2017) (PubMed).

Stadem, Hilgers, Bengo, Cusick, Ndidde, Slusher, Lund: "Markers of oxidative stress in umbilical cord blood from G6PD deficient African newborns." in: **PLoS ONE**, Vol. 12, Issue 2, pp. e0172980, (2017) (PubMed).

Mostek, Dietrich, Słowińska, Ciereszko: "Cryopreservation of bull semen is associated with carbonylation of sperm proteins." in: **Theriogenology**, Vol. 92, pp. 95-102, (2017) (PubMed).

Francioli, Wang, Parapanov, Abdelnour, Lugrin, Gronchi, Perentes, Eckert, Ris, Piquilloud, Krueger, Liaudet: "Pyrrolidine dithiocarbamate administered during ex-vivo lung perfusion promotes rehabilitation of injured donor rat lungs obtained after prolonged warm ischemia." in: **PLoS ONE**, Vol. 12, Issue 3, pp. e0173916, (2017) (PubMed).

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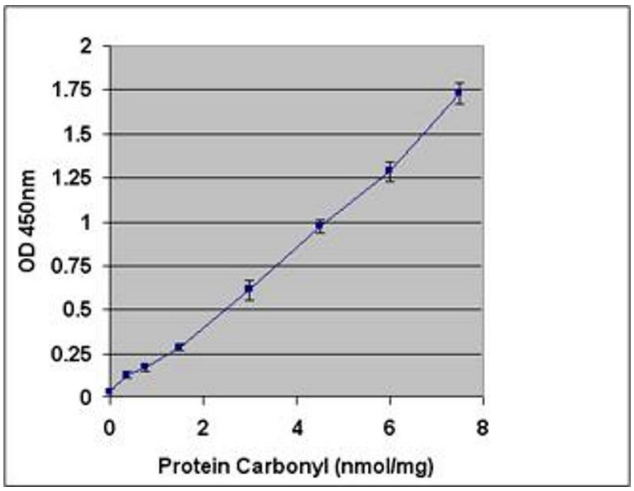

ELISA

Image 1. Standard Curve Generated with the OxiSelect™ Protein Carbonyl ELISA Kit.