Revised Protocol

Product Manual

OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)

Catalog Number

STA-320	96 assays
STA-320-5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Free radicals and other reactive species are constantly generated *in vivo* and cause oxidative damage to biomolecules, a process held in check only by the existence of multiple antioxidant and repair systems as well as the replacement of damaged nucleic acids, proteins and lipids. DNA is probably the most biologically significant target of oxidative attack, and it is widely thought that continuous oxidative damage to DNA is a significant contributor to the age-related development of the major cancers, such as those of the colon, breast, rectum, and prostate. Among numerous types of oxidative DNA damage, the formation of 8-hydroxydeoxyguanosine (8-OHdG) is a ubiquitous marker of oxidative stress. 8-OHdG, one of the oxidative DNA damage byproducts, is physiologically formed and enhanced by chemical carcinogens. During the repair of damaged DNA *in vivo* by exonucleases, the resulting 8-OH-dG is excreted without further metabolism into urine.

Cell Biolabs' Oxidative DNA Damage ELISA Kit is a competitive enzyme immunoassay developed for rapid detection and quantitation of 8-OHdG in urine, serum, or other cell or tissue DNA samples. The quantity of 8-OHdG in unknown sample is determined by comparing its absorbance with that of a known 8-OHdG standard curve. The kit has an 8-OHdG detection sensitivity range of 100 pg/mL to 20 ng/mL. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown samples.

Assay Principle

The Oxidative DNA Damage ELISA kit is a competitive ELISA for the quantitative measurement of 8-OHdG. The unknown 8-OHdG samples or 8-OHdG standards are first added to an 8-OHdG/BSA conjugate preabsorbed EIA plate. After a brief incubation, an anti-8-OHdG monoclonal antibody is added, followed by an HRP conjugated secondary antibody. The 8-OHdG content in unknown samples is determined by comparison with predetermined 8-OHdG standard curve.

Related Products

1. STA-303: OxiSelect™ Nitrotyrosine Immunoblot Kit
2. STA-305: OxiSelect™ Nitrotyrosine ELISA Kit
3. STA-308: OxiSelect™ Protein Carbonyl Immunoblot Kit
4. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
5. STA-315: OxiSelect™ Protein Carbonyl Spectrophotometric Assay
6. STA-330: OxiSelect™ TBARS Assay Kit (MDA Quantitation)
7. STA-332: OxiSelect™ MDA ELISA Kit
8. STA-334: OxiSelect™ HNE Adduct ELISA Kit
9. STA-324: OxiSelect™ Oxidative DNA Damage Quantitation Kit (AP Sites)
10. STA-325: OxiSelect™ Oxidative RNA Damage ELISA Kit (8-OHG)

Kit Components

Box 1 (shipped at room temperature)

1. 96-well Protein Binding Plate (Part No. 231001): One strip well 96-well plate.
2. Anti-8-OHdG Antibody (Part No. 232002): One 15 µL vial of anti-8-OHdG.
3. Secondary Antibody, HRP Conjugate (Part No. 10902): One 50 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. 8-OHdG Standard (Part No. 232003): One 100 µL vial of 2 µg/mL 8-OHdG in 1X PBS, 0.1% BSA.

Box 2 (shipped on blue ice packs)

1. 8-OHdG Conjugate (Part No. 232001): One 20 µL vial of 8-OHdG-BSA conjugate at 1.0 mg/mL in PBS.

Materials Not Supplied

1. 8-OHdG samples such as serum, urine, cell or tissue DNA
2. DNA Extraction Kit
3. Sodium Acetate, pH 5.2
4. Tris Buffer, pH7.5
5. Nuclease P1, Alkaline Phosphatase
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 **8-OHdG Standard at -20ºC** and the **8-OHdG Conjugate at -80ºC** to avoid multiple freeze/thaw cycles. Store all other components at 4ºC until their expiration dates.

Preparation of Reagents

- 8-OHdG Coated Plate: Dilute the proper amount of 8-OHdG Conjugate (1 mg/mL) to **1 µg/mL** in 1X PBS. Add 100 µL of the **1 µg/mL** 8-OHdG Conjugate to each well and incubate overnight at 4ºC. Remove the 8-OHdG coating solution and wash once with dH_2O. Blot plate on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1 hr at room temperature. Transfer the plate to 4ºC and remove the Assay Diluent immediately before use.
  Note: 8-OHdG coated plate is not stable. We recommend using it within 24 hrs after coating.
- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Anti-8-OHdG Antibody and Secondary Antibody: Immediately before use dilute the Anti-8-OHdG Antibody 1:500 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Prepare a dilution series of 8-OHdG standards in the concentration range of 0 ng/mL – 20 ng/mL by diluting the 8-OHdG Standard in Assay Diluent (Table 1).

Standard Tubes	8-OHdG Standard (µL)	Assay Diluent (µL)	8-OHdG (ng/mL)
1	10	990	20
2	500 of Tube #1	500	10
3	500 of Tube #2	500	5
4	500 of Tube #3	500	2.5
5	500 of Tube #4	500	1.25
6	500 of Tube #5	500	0.625
7	500 of Tube #6	500	0.313
8	500 of Tube #7	500	0.156
9	500 of Tube #8	500	0.078
10	0	500	0

Table 1. Preparation of 8-OHdG Standards

Preparation of Samples

I. Urine or Serum Samples

Clear urine or serum samples can be diluted in Assay Diluent and used directly in the assay. Samples containing precipitates should be centrifuged at 3000 g for 10 minutes, or filtered through 0.45 µm filter, prior to use in the assay.

II. Cell or Tissue DNA Samples:

1. Extract DNA from cell or tissue samples by a desired method or commercial DNA Extraction kit.
2. Dissolve extracted DNA in water at 1-5 mg/mL.
3. Convert DNA sample to single-stranded DNA by incubating the sample at 95ºC for 5 minutes and rapidly chilling on ice.
4. Digest DNA sample to nucleosides by incubating the denatured DNA with 5-20 units of nuclease P1 for 2 hrs at 37ºC in 20 mM Sodium Acetate, pH 5.2, and following with treatment of 5-10 units of alkaline phosphatase for 1 hr at 37 ^{0}C in 100 mM Tris, pH 7.5.
5. The reaction mixture is centrifuged for 5 minutes at 6000 g and the supernatant is used for the 8-OHdG ELISA assay.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each 8-OHdG sample including unknown and standard should be assayed in duplicate. High content 8-OHdG urine or serum samples should be diluted at least 10-20 fold in Assay Diluent.
2. Add 50 µL of unknown sample or 8-OHdG standard to the wells of the 8-OHdG Conjugate coated plate. Incubate at room temperature for 10 minutes on an orbital shaker.
3. Add 50 µL of the diluted anti-8-OHdG antibody to each well, incubate at room temperature for 1 hour on an orbital shaker.
4. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well 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.
5. Add 100 µL of the diluted Secondary Antibody-Enzyme Conjugate to all wells.
6. Incubate at room temperature for 1 hour on an orbital shaker.
7. Wash microwell strips 3 times according to step 4 above. Proceed immediately to the next step.
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 into each well, including the blank wells. Results should be read immediately (color will fade over time).

10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

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

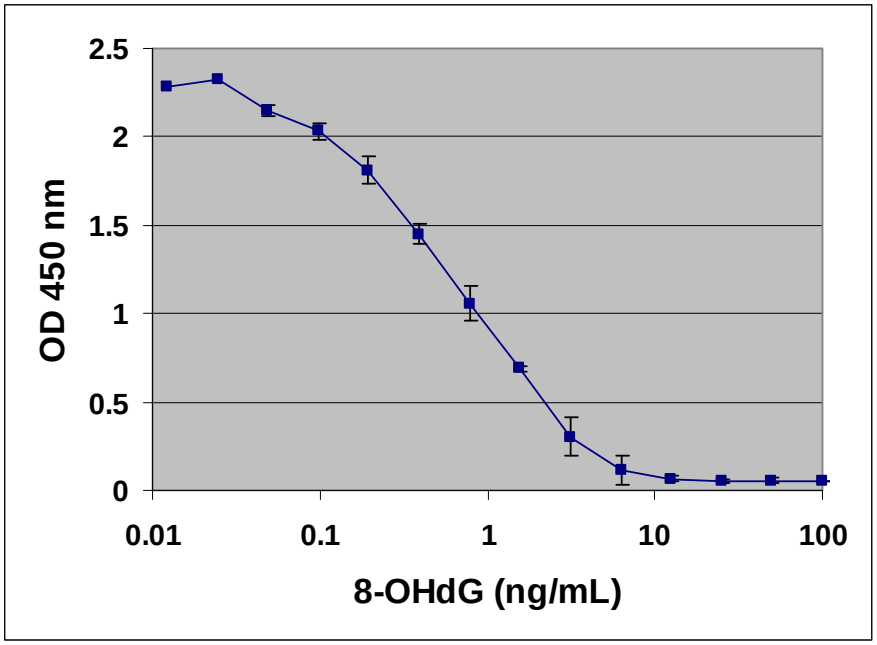

Figure 1: 8-OHdG ELISA Standard Curve.

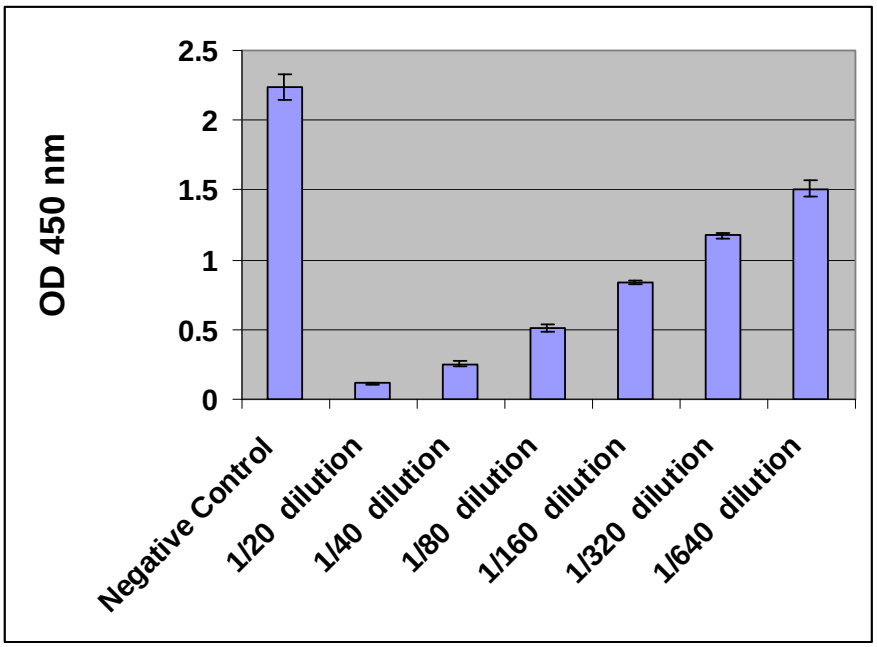

Figure 2: 8-OHdG level in human urine sample.

References

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2. Shen J, Deininger P, Hunt J. D, and Zhao H. (2007) Cancer **109**, 574-580.
3. Wu L. L, Chiou C. C, Chang P. Y and Wu J. T. (2004) Clin Chim Acta. **339**, 1-9.

Recent Product Citations

1. Ksiazek, K. et al. (2008). Impaired response to oxidative stress in senescent cells may lead to acccumulation of DNA damage in mesothelial cells from aged donors. *Biochem. and Biophys. Res. Comm.* **373**:335-339.
2. Rao, M. et al. (2008). Mitochondrial DNA injury and mortality in hemodialysis patients. *J. Am. Soc. Nephrol.* **20**:189-196.
3. Ksiazek, K. et al. (2008). Vulnerability to oxidative stress and differen patterns of senescence in human peritoneal mesothelial cell strains. *Am J. Physiol. Regulatory Integrative Comp. Physiol.* **296**:R374-R382.
4. Hasegawa, T. et al. (2009*). Suppression of nitrosative and oxidative stress to reduce cardiac allograft vasculopathy. Am.J. Physiol. Hear Circ. Physiol.* 10.1152/ajpheart.00498.2008.
5. Pialoux, V. et al. (2009). Effects of exposure to intermittent hypoxia on oxidative stress and acute hypoxic ventilatory response in humans. *Am. J. Respir. Crit. Care Med.* 10.1164/rccm.200905-0671OC.

Warranty

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