Product Manual

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

Catalog Number

STA-320-T **32 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.

The OxiSelect™ 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 Trial Size DNA Damage ELISA Kit provides sufficient reagents to perform up to 32 assays, including standard curve and unknown samples.

Assay Principle

The OxiSelect™ 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 microplate. 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-321: OxiSelect™ DNA Double-Strand Break (DSB) Staining Kit
2. STA-324: OxiSelect™ Oxidative DNA Damage Quantitation Kit (AP sites)
3. STA-325: OxiSelect™ Oxidative RNA Damage ELISA Kit (8-OHG Quantitation)
4. STA-350: OxiSelect™ Comet Assay Kit (3-Well Slides), 15 Assays
5. STA-355: OxiSelect™ 96-Well Comet Assay Kit

Kit Components

Box 1 (shipped at room temperature)

1. Protein Binding Strip Well Plate (Part No. 231001-T): One strip-well microplate containing 32 wells (8 x 4).
2. Anti-8-OHdG Antibody (Part No. 232002-T): One 5 µL vial of anti-8-OHdG.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 230003): One 20 µL vial.
4. Assay Diluent (Part No. 310804-T): One 20 mL bottle.
5. 10X Wash Buffer (Part No. 310806-T): One 30 mL bottle.
6. Substrate Solution (Part No. 310807-T): One 4 mL amber bottle.
7. Stop Solution (Part. No. 310808-T): One 4 mL bottle.
8. 8-OHdG Standard (Part No. 232003-T): One 30 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-T): One 5 µL vial of 8-OHdG-BSA conjugate at 1.0 mg/mL in PBS.

Materials Not Supplied

1. 8-OHdG samples such as serum, plasma, urine, or DNA extracted from cells or tissues
2. DNA Extraction Kit
3. Sodium Acetate, pH 5.2
4. Tris Buffer, pH7.5
5. Nuclease P1, Alkaline Phosphatase
6. 10 kDa molecular weight cutoff (MWCO) centrifuge spin filter (e.g. Amicon Ultra 0.5mL)

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.

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: The 8-OHdG coated wells are not stable and should be used within 24 hrs after coating. Only coat the number of wells to be used immediately.

- 1X Wash Buffer: Dilute the 10X Wash Buffer 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 to 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, Plasma or Serum Samples

Clear urine, plasma 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.

Note: All mouse and rat serum and plasma samples must be filtered using a 10kDa spin filter prior to testing.

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 (previously reconstituted in the manufacturer's recommended buffer) for 2 hrs at 37ºC in a final concentration of 20 mM Sodium Acetate, pH 5.2.
5. Add 5-10 units of alkaline phosphatase (previously reconstituted in the manufacturer's recommended buffer) plus sufficient Tris buffer to a final concentration of 100 mM Tris, pH 7.5, and incubate for 1 hr at 37ºC.
6. Centrifuge the reaction mixture for 5 minutes at 6000 x g and collect the supernatant for use in the ELISA.

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.

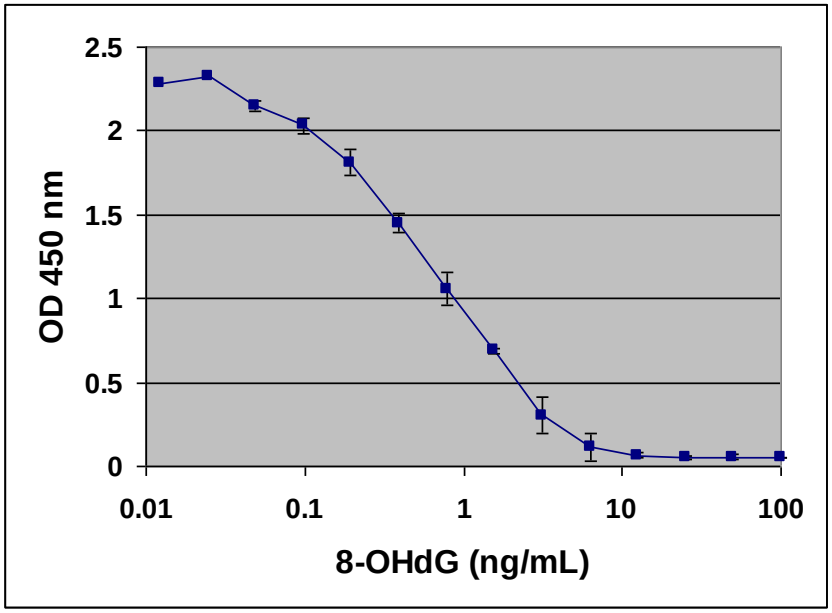

Figure 1: 8-OHdG ELISA Standard Curve.

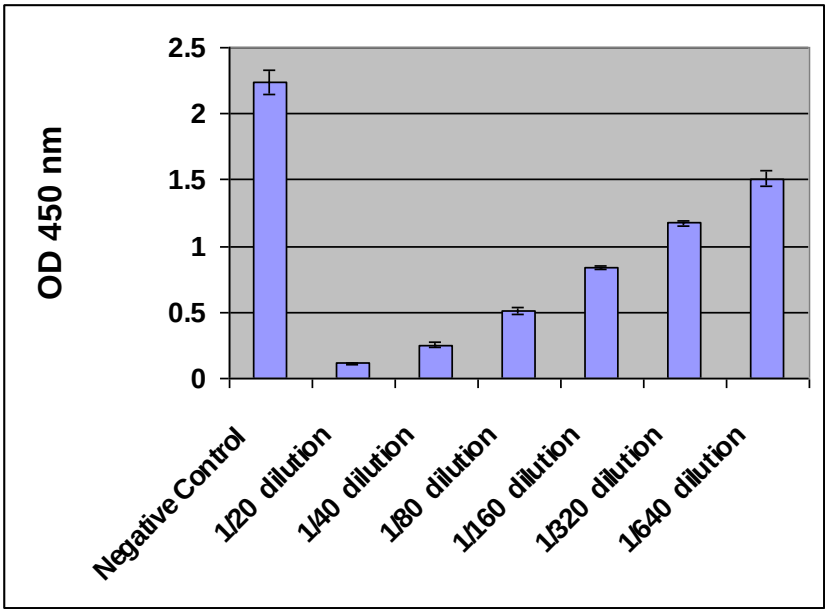

Figure 2: 8-OHdG level in human urine sample.

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Recent Product Citations

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