Revised Protocol

Product Manual

OxiSelect™ Oxidative RNA Damage ELISA Kit (8-OHG Quantitation)

Catalog Number

STA-325	96 assays
STA-325-5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
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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 nuclei acids, proteins and lipids. RNA and DNA are probably the most biologically significant targets of oxidative attack. Recently, oxidative damage to RNA molecules has been described in several neurological diseases including Alzheimer's disease, Parkinson's disease, Down syndrome, dementia with Lewy bodies, prion disease, subacute sclerosing panencephalitis, and xeroderma pigmentosum. Among numerous types of RNA oxidative damage, the formation of 8-hydroxyguanosine (8-OHG) is a ubiquitous marker of oxidative stress.

The OxiSelect™ Oxidative RNA Damage ELISA Kit is a competitive enzyme immunoassay developed for rapid detection and quantitation of 8-OHG in urine, serum, cerebrospinal fluid or other cell or tissue RNA samples. The quantity of 8-OHG in unknown sample is determined by comparing its absorbance with that of a known 8-OHG standard curve. The kit has an 8-OHG detection sensitivity range of 300 pg/mL to 40 ng/mL. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown samples.

Assay Principle

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

Related Products

1. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
2. STA-321: OxiSelect™ DNA Double-Strand Break (DSB) Staining Kit
3. STA-324: OxiSelect™ Oxidative DNA Damage Quantitation Kit (AP sites)
4. STA-350: OxiSelect™ Comet Assay Kit (3-Well Slides), 15 Assays
5. STA-351: OxiSelect™ Comet Assay Kit (3-Well Slides), 75 Assays

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-OHG Antibody (Part No. 232502): One 15 µL vial of anti-8-OHG.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 230003): One 20 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-OHG Standard (Part No. 232503): One 100 µL vial of 2 µg/mL 8-OHG in 1X PBS, 0.1% BSA.

Box 2 (shipped on blue ice packs)

1. 8-OHG Conjugate (Part No. 232501): One 20 µL vial of 8-OHG-BSA conjugate at 1.0 mg/mL in PBS.

Materials Not Supplied

1. 8-OHG samples such as serum, plasma, urine, cerebrospinal fluid, or RNA extracted from cells or tissues
2. RNA Extraction Kit
3. Sodium Acetate, pH 5.2
4. Tris Buffer, pH 7.5
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-OHG Standard at -20ºC and the 8-OHG Conjugate at -80ºC to avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

Preparation of Reagents

- 8-OHG Coated Plate: Dilute the proper amount of 8-OHG Conjugate (1 mg/mL) to **1 µg/mL** in 1X PBS. Add 100 µL of the **1 µg/mL** 8-OHG Conjugate to each well and incubate overnight at 4ºC. Remove the 8-OHG 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-OHG 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-OHG Antibody and Secondary Antibody: Immediately before use dilute the Anti-8-OHG 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-OHG standards in the concentration range of 0 ng/mL – 40 ng/mL by diluting the 8-OHG Standard in Assay Diluent (Table 1).

Standard Tubes	8-OHG Standard (µL)	Assay Diluent (µL)	8-OHG (ng/mL)
1	20	980	40
2	500 of Tube #1	500	20
3	500 of Tube #2	500	10
4	500 of Tube #3	500	5
5	500 of Tube #4	500	2.5
6	500 of Tube #5	500	1.25
7	500 of Tube #6	500	0.625
8	500 of Tube #7	500	0.313
9	500 of Tube #8	500	0.156
10	0	500	0

Table 1. Preparation of 8-OHG Standards

Preparation of Samples

I. Urine, Serum, Plasma, or Cerebrospinal Fluid Samples (Quantitation of 8-OHG and 8-OHdG)

Clear urine, serum or cerebrospinal fluid 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 RNA Samples (Quantitation of 8-OHG):

1. Extract RNA from cell or tissue samples by a desired method or commercial RNA Extraction kit.
2. Dissolve extracted RNA in water at 1-5 mg/mL.
3. Digest RNA sample to nucleosides by incubating the sample 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.
4. The reaction mixture is centrifuged for 5 minutes at 6000 g and the supernatant is used for the 8-OHG assay.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each 8-OHG sample including unknown and standard should be assayed in duplicate. High content 8-OHG samples should be diluted with Assay Diluent.
2. Add 50 µL of unknown sample or 8-OHG standard to the wells of the 8-OHG Conjugate coated plate. Incubate at room temperature for 10 minutes on an orbital shaker.

3. Add 50 µL of the diluted anti-8-OHG 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 RNA Damage ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

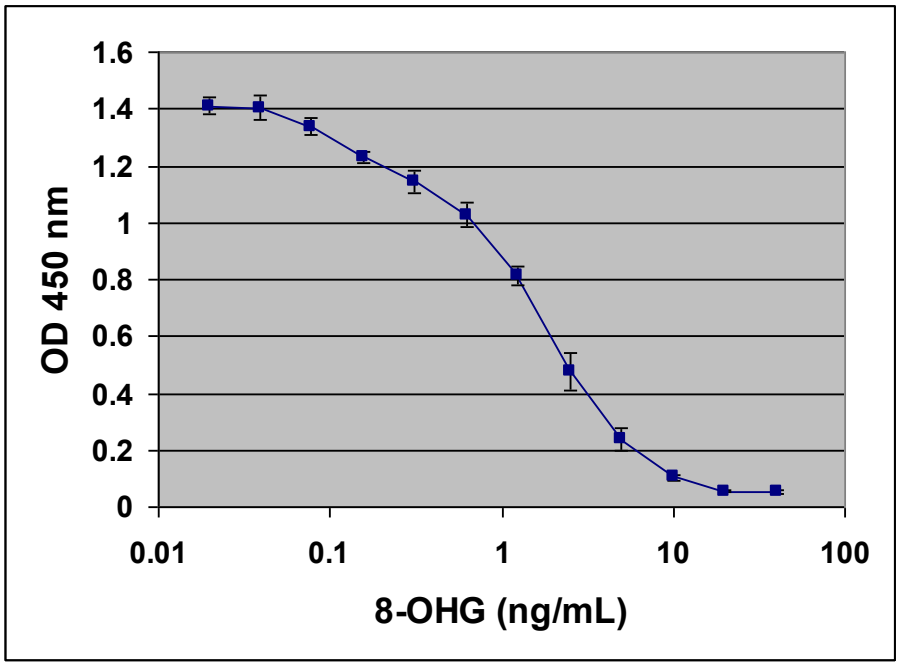

Figure 1: 8-OHG ELISA Standard Curve.

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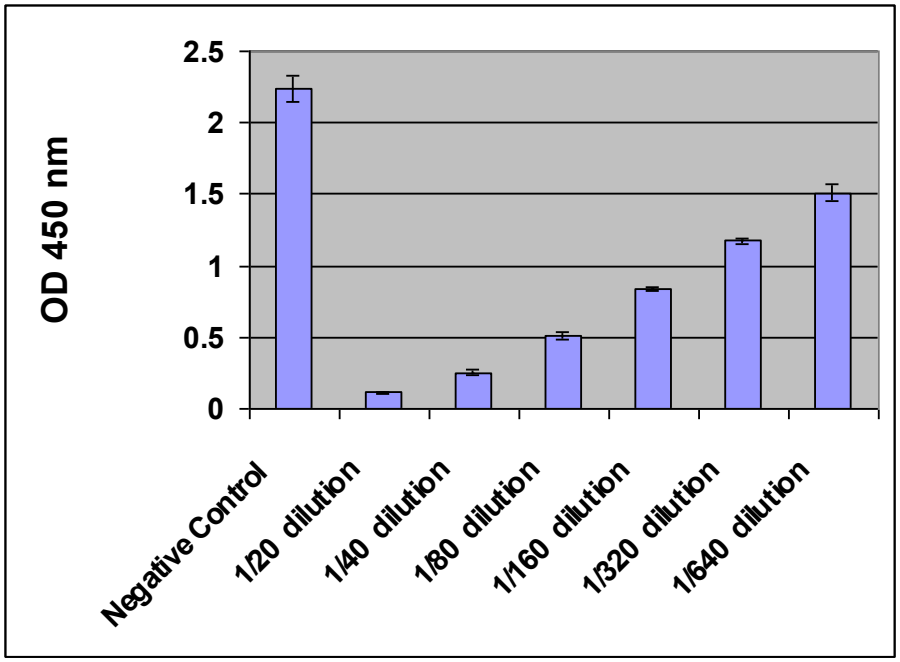

Figure 2: 8-OHG and 8-OHdG level in human urine sample.

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Recent Product Citations

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