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.

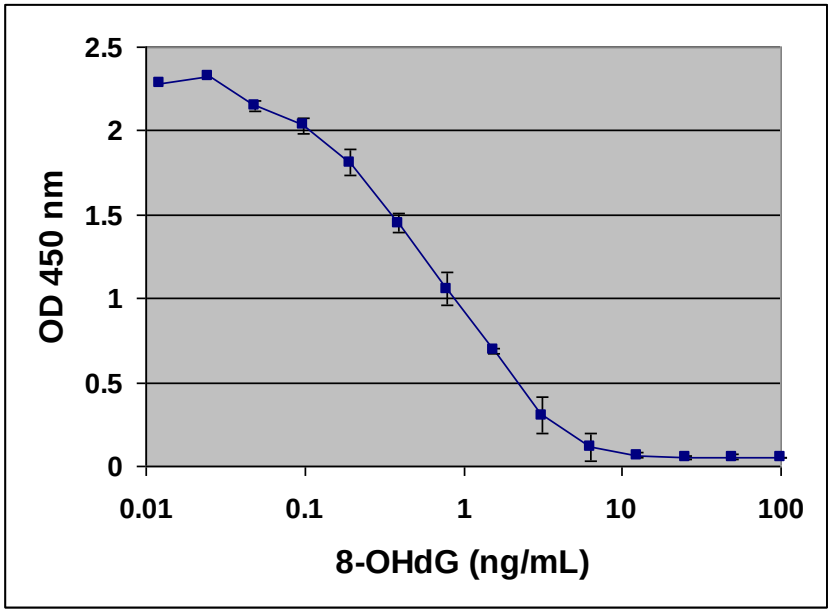

Figure 1: 8-OHdG ELISA Standard Curve.

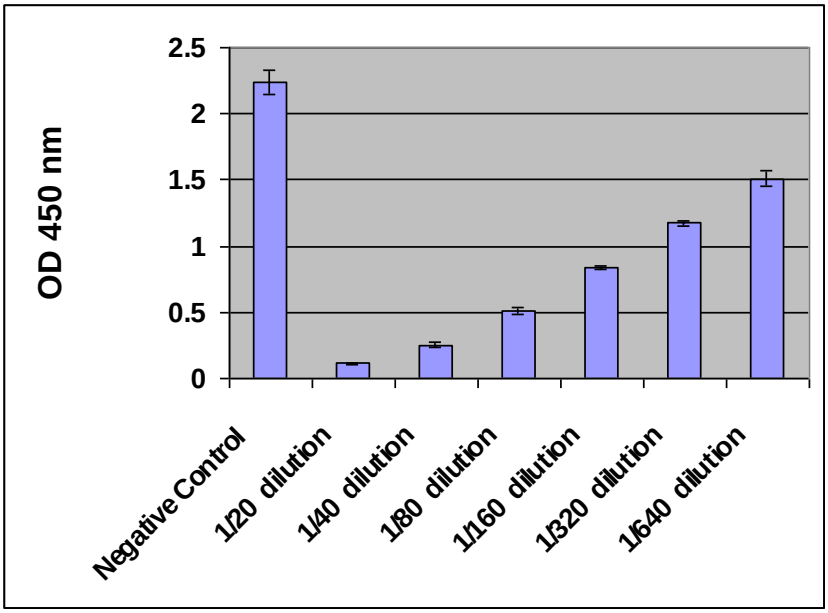

Figure 2: 8-OHdG level in human urine sample.

References

1. Patel P. R, Bevan R. J, Mistry N and Lunec J. (2007) J. Free Radic Biol Med. **42**, 552-558.
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. Dewidar, B. et al. (2023). Alterations of hepatic energy metabolism in murine models of obesity, diabetes and fatty liver diseases. *EBioMedicine*. **94**:104714. doi: 10.1016/j.ebiom.2023.104714.

2. Linillos-Pradillo, B. et al. (2023). Low Dose of BPA Induces Liver Injury through Oxidative Stress, Inflammation and Apoptosis in Long-Evans Lactating Rats and Its Perinatal Effect on Female PND6 Offspring. *Int J Mol Sci*. **24**(5):4585. doi: 10.3390/ijms24054585.
3. Hadžić, Z. et al. (2023). Oxidative Stress and C-Reactive Protein as Salivary Biomarkers in Smokers with Periodontitis Stage III and IV After Non-Surgical Periodontal Therapy (A Pilot Study). *Acta Med. Mediterr*. **39**:947-953. doi: 10.19193/0393-6384_2023_4_131.
4. Ivanova, I. et al. (2023). UVA-induced metabolic changes in non-malignant skin cells and the potential role of pyruvate as antioxidant. *Photochem Photobiol Sci*. doi: 10.1007/s43630-023-00419-z.
5. Peinado, V.I. et al. (2023). Atrophy signaling pathways in respiratory and limb muscles of guinea pigs exposed to chronic cigarette smoke: role of soluble guanylate cyclase stimulation. *Am J Physiol Lung Cell Mol Physiol*. **324**(5):L677-L693. doi: 10.1152/ajplung.00258.2022.
6. Xiao, L. et al. (2023). Autism-like behavior of murine offspring induced by prenatal exposure to progestin is associated with gastrointestinal dysfunction due to claudin-1 suppression. *FEBS J*. doi: 10.1111/febs.16761.
7. Miao, N. et al. (2023). Oxidized mitochondrial DNA induces gasdermin D oligomerization in systemic lupus erythematosus. *Nat Commun*. **14**(1):872. doi: 10.1038/s41467-023-36522-z.
8. Rodriguez-Pérez, M.D. et al. (2023). The Effect of the Extra Virgin Olive Oil Minor Phenolic Compound 3',4'-Dihydroxyphenylglycol in Experimental Diabetic Kidney Disease. *Nutrients*. **15**(2):377. doi: 10.3390/nu15020377.
9. Chhunchha, B. et al. (2023). Hydralazine Revives Cellular and Ocular Lens Health-Span by Ameliorating the Aging and Oxidative-Dependent Loss of the Nrf2-Activated Cellular Stress Response. *Antioxidants (Basel)*. **12**(1):140. doi: 10.3390/antiox12010140.
10. Ko, E.J. et al. (2022). Effect of dual inhibition of DPP4 and SGLT2 on tacrolimus-induced diabetes mellitus and nephrotoxicity in a rat model. *Am J Transplant*. doi: 10.1111/ajt.17035.
11. Hu, B. et al. (2022). Repurposing Ivermectin to augment chemotherapy's efficacy in osteosarcoma. *Hum Exp Toxicol*. doi: 10.1177/09603271221143693.
12. Park, C. et al. (2022). Phloroglucinol Attenuates DNA Damage and Apoptosis Induced by Oxidative Stress in Human Retinal Pigment Epithelium ARPE-19 Cells by Blocking the Production of Mitochondrial ROS. *Antioxidants (Basel)*. **11**(12):2353. doi: 10.3390/antiox11122353.
13. Wang, Y. et al. (2022). Protective effect of hydroxysafflor yellow A on renal ischemia-reperfusion injury by targeting the Akt-Nrf2 axis in mice. *Exp Ther Med*. doi: 10.3892/etm.2022.11677.
14. Wang, B. et al. (2022). Let-7e-5p, a promising novel biomarker for benzene toxicity, is involved in benzene-induced hematopoietic toxicity through targeting caspase-3 and p21. *Ecotoxicol Environ Saf*. doi: 10.1016/j.ecoenv.2022.114142.
15. Ibrahim, M.A. et al. (2022). Bone-Marrow-Derived Mesenchymal Stem Cells, Their Conditioned Media, and Olive Leaf Extract Protect against Cisplatin-Induced Toxicity by Alleviating Oxidative Stress, Inflammation, and Apoptosis in Rats. *Toxics*. **10**(9):526. doi: 10.3390/toxics10090526.
16. Wang, H. et al. (2022). Biphasic effects of statins on neuron cell functions under oxygen-glucose deprivation and normal culturing conditions via different mechanisms. *Pharmacol Res Perspect*. **10**(5): e01001. doi: 10.1002/prp2.1001.
17. Ohira, H. et al. (2022). Suppression of colonic oxidative stress caused by chronic ethanol administration and attenuation of ethanol-induced colitis and gut leakiness by oral administration of sesaminol in mice. *Food Funct*. doi: 10.1039/d1fo04120g.

18. Fujita, N. et al. (2022). Association of oxidative stress with erectile dysfunction in community-dwelling men and men on dialysis. *Aging Male*. **25**(1):193-201. doi: 10.1080/13685538.2022.2103113.
19. Del Mar Rivas-Chacón, L. et al. (2022). Preventive Effect of Cocoa Flavonoids via Suppression of Oxidative Stress-Induced Apoptosis in Auditory Senescent Cells. *Antioxidants (Basel)*. **11**(8):1450. doi: 10.3390/antiox11081450.
20. Konieczka, P. et al. (2022). Increased arginine, lysine, and methionine levels can improve the performance, gut integrity and immune status of turkeys but the effect is interactive and depends on challenge conditions. *Vet Res*. **53**(1):59. doi: 10.1186/s13567-022-01080-7.
21. Muhammed, S. et al. (2022). The Effect of Zingiber, Alpinia Officinarum with Periodontal Therapy on Clinical Outcome and Oxidative Stress. *J. Hunan Univ. Nat. Sci*. **49**(6):32-43. doi: 10.55463/issn.1674-2974.49.6.4.
22. Dworzański, W. et al. (2022). Oxidative, epigenetic changes and fermentation processes in the intestine of rats fed high-fat diets supplemented with various chromium forms. *Sci Rep*. **12**(1):9817. doi: 10.1038/s41598-022-13328-5.
23. Ghamry, H.I. et al. (2022). Ginseng® Alleviates Malathion-Induced Hepatorenal Injury through Modulation of the Biochemical, Antioxidant, Anti-Apoptotic, and Anti-Inflammatory Markers in Male Rats. *Life (Basel)*. **12**(5):771. doi: 10.3390/life12050771.
24. Pérez-Soto, E. et al. (2022). High-Risk HPV with Multiple Infections Promotes CYP2E1, Lipoperoxidation and Pro-Inflammatory Cytokines in Semen of Asymptomatic Infertile Men. *Antioxidants*. **11**(6):1051. doi: 10.3390/antiox11061051.
25. El Okle, O.S. et al. (2022). Ornipural® Mitigates Malathion-Induced Hepato-Renal Damage in Rats via Amelioration of Oxidative Stress Biomarkers, Restoration of Antioxidant Activity, and Attenuation of Inflammatory Response. *Antioxidants (Basel)*. **11**(4):757. doi: 10.3390/antiox11040757.
26. Maciejczyk, M. et al. (2022). α-Lipoic Acid Strengthens the Antioxidant Barrier and Reduces Oxidative, Nitrosative, and Glycative Damage, as well as Inhibits Inflammation and Apoptosis in the Hypothalamus but Not in the Cerebral Cortex of Insulin-Resistant Rats. *Oxid Med Cell Longev*. doi: 10.1155/2022/7450514.
27. Maciejczyk, M. et al. (2022). Oxidation, Glycation, and Carbamylation of Salivary Biomolecules in Healthy Children, Adults, and the Elderly: Can Saliva Be Used in the Assessment of Aging? *J Inflamm Res*. **15**:2051-2073. doi: 10.2147/JIR.S356029.
28. Kim, Y.B. et al. (2022). Incorporation of Dietary Methyl Sulfonyl Methane into the Egg Albumens of Laying Hens. *Antioxidants (Basel)*. **11**(3):517. doi: 10.3390/antiox11030517.
29. Ivanova, I. et al. (2022). Investigation of the HelioVital filter foil revealed protective effects against UVA1 irradiation-induced DNA damage and against UVA1-induced expression of matrixmetalloproteinases (MMP) MMP1, MMP2, MMP3 and MMP15. *Photochem Photobiol Sci*. doi: 10.1007/s43630-022-00177-4.
30. Tungmunnithum, D. et al. (2022). Flavonoids from Sacred Lotus Stamen Extract Slows Chronological Aging in Yeast Model by Reducing Oxidative Stress and Maintaining Cellular Metabolism. *Cells*. **11**(4):599. doi: 10.3390/cells11040599.

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