Datasheet for ABIN2344960

Oxidative DNA Damage ELISA Kit

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Overview

Quantity:	96 tests
Target:	Oxidative DNA Damage
Reactivity:	Others
Method Type:	Competition ELISA
Application:	ELISA

Product Details

Purpose:	The OxiSelect™ Oxidative DNA Damage ELISA kit is a competitive ELISA for the quantitative measurement of 8-OHdG.
Brand:	OxiSelect™
Sample Type:	Urine, Plasma, Cell Samples, Serum, Tissue Samples
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Sensitivity:	100 pg/mL
Characteristics:	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 kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown samples.

Product Details

Components:	1. 96-well Protein Binding Plate : One strip-well 96 well microplate. 2. Anti-8-OHdG Antibody : One 15 µL vial of anti-8-OHdG. 3. Secondary Antibody, HRP Conjugate (1000X) : One 20 µL vial. 4. Assay Diluent : One 50 mL bottle. 5. 10X Wash Buffer : One 100 mL bottle. 6. Substrate Solution : One 12 mL amber bottle. 7. Stop Solution (Part. No. 310808): One 12 mL bottle. 8. 8-OHdG Standard : One 100 µL vial of 2 µg/mL 8-OHdG in 1X PBS, 0.1% BSA. Box 2 (shipped on blue ice packs)
Material not included:	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, pH 7.5 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)

Target Details

Target:	Oxidative DNA Damage
Background:	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.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Detect as little as 100 pg/mL of 8-OHdG

Application Details

	• Suitable for use with urine, serum, cells or tissues • 8-OHdG standard included for absolute quantitation
Plate:	Uncoated
Protocol:	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.
Reagent Preparation:	• 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 3 overnight at 4 °C. Remove the 8-OHdG coating solution and wash once with dH2O. 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 4 °C 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). Assay Diluent 8-OHdG Standard Tubes 8-OHdG Standard (µL) (µL) (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
Sample Preparation:	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: For mouse or rat serum or plasma samples it is highly recommended to filter the sample with a 10 kDa spin filter prior to testing. 4 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 95 °C 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 37 °C in a final concentration of 20 mM Sodium Acetate, pH 5.2, followed by treatment of 5-10 units of alkaline phosphatase for 1 hr at 37 °C in a final concentration of 100 mM Tris, pH 7.5.

Application Details

	5. The reaction mixture is centrifuged for 5 minutes at 6000 g and the supernatant is used for the 8- OHdG ELISA assay.
Assay Procedure:	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. 5 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.
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid multiple freeze/thaw cycles.
Storage:	-20 °C/-80 °C
Storage Comment:	Upon receipt, aliquot and store the 8-OHdG Standard at -20°C and the 8-OHdG Conjugate at -80°C to avoid multiple freeze/thaw cycles. Store all other components at 4°C.

Publications

Product cited in:	Loperfido, Jarmin, Dastidar, Di Matteo, Perini, Moore, Nair, Samara-Kuko, Athanasopoulos, Tedesco, Dickson, Sampaolesi, VandenDriessche, Chuah: "piggyBac transposons expressing full-length human dystrophin enable genetic correction of dystrophic mesoangioblasts." in: **Nucleic acids research**, Vol. 44, Issue 2, pp. 744-60, (2016) (PubMed).

Publications

Li, Liu, Li, Sun, Xu, Xie, Zhang: "PTPRR regulates ERK dephosphorylation in depression mice model." in: **Journal of affective disorders**, Vol. 193, pp. 233-41, (2016) (PubMed).

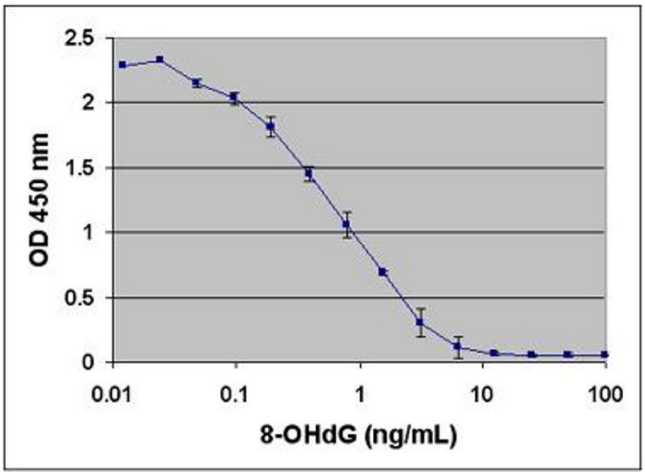
Madison, Roller, Okeoma: "Human semen contains exosomes with potent anti-HIV-1 activity." in: **Retrovirology**, Vol. 11, pp. 102, (2015) (PubMed).

Rohrbach, Jarboe, Anderson, Trummell, Hicks, Weaver, Yang, Oster, Deshane, Steele, Siegal, Bonner, Willey: "Targeting the effector domain of the myristoylated alanine rich C-kinase substrate enhances lung cancer radiation sensitivity." in: **International journal of oncology**, Vol. 46, Issue 3, pp. 1079-88, (2015) (PubMed).

Noh, Maze, Zhao, Xiang, Wenderski, Lewis, Shen, Li, Allis: "ATRX tolerates activity-dependent histone H3 methyl/phos switching to maintain repetitive element silencing in neurons." in: **Proceedings of the National Academy of Sciences of the United States of America**, Vol. 112, Issue 22, pp. 6820-7, (2015) (PubMed).

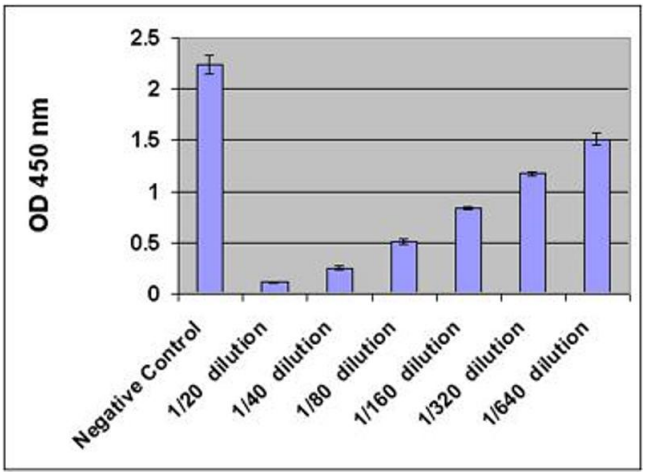
There are more publications referencing this product on: Product page

Images



ELISA

Image 1. 8-OHdG ELISA Standard Curve



ELISA

Image 2. 8-OHdG Levels in Human Urine.