



PicoKine™ ELISA

Catalog number: EK7114

For the quantitation of **All Animals** concentrations in Cell lysates, Plasma, Sample matrices, Urine

This package insert must be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.

DNA Damage (8-OHdG) ELISA Kit

Catalog Number: EK7114

Introduction

Boster's ELISA Kit is a competitive assay that can be used for the quantification of 8-OHdG in urine, cell culture, plasma, and other sample matrices. The ELISA utilizes an 8-hydroxy-2-deoxy Guanosine-coated plate and an HRP-conjugated antibody for detection which allows for an assay range of 0.94 - 60 ng/mL, with a sensitivity of 0.59 ng/mL. The other highlights of this kit are a quick incubation time of 60 minutes, stable reagents, and an easy to use protocol. It is important to note that the 8-OHdG antibody used in this assay recognizes both free 8-OHdG and DNA-incorporated 8-OHdG. Since complex samples such as plasma, cell lysates, and tissues are comprised of mixtures of DNA fragments and free 8-OHdG, concentrations of 8-OHdG reported by ELISA methodology will not coincide with those reported by LC-MS where the single nucleoside is typically measured. This should be kept in mind when analyzing and interpreting experimental results.

Overview

Product Name	DNA Damage (8-OHdG) ELISA Kit
Reactive Species	All Animals
Size	96wells/kit, with removable strips.
Description	Colorimetric detection of DNA Damage (8-OHdG). 96wells/kit, with removable strips.
Sensitivity	0.59 ng/mL *The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.
Detection Range	0.94 - 60 ng/mL
Storage Instructions	Store at 4°C and -20°C. (All reagents are stable as supplied at 4°C, except the standard , which should be stored at -20°C.)
Cross Reactivity	8-Hydroxy-2-deoxy Guanosine (8-OHdG): 100%. 8-Hydroxy Guanosine (8-OHG): 23%. 8-Hydroxy Guanine (8-oxoG): 23%. Guanosine: <0.01%.
Precision	Intra-Assay Precision: Three samples of known concentration were assayed thirty times on one plate; the intra-assay coefficient of variation of the DNA Damage ELISA has been determined to be <5%. Inter-Assay Precision: Three samples of known concentration were assayed thirty times in three individual assays; the inter-assay coefficient of variation of the DNA Damage ELISA has been determined to be <5%.
Incubation Time	1 hour
Number Of Samples	39 samples in duplicate

Kit Components/Materials Provided

Description	Quantity
8-hydroxy-2-deoxy Guanosine : BSA Coated Plate	1 Plate
8-hydroxy-2-deoxy Guanosine Standard	1 vial/ 100uL
8-hydroxy-2-deoxy Guanosine HRP Conjugated Monoclonal Antibody	1 vial/75uL
Sample and Standard Diluent	1 vial/50mL
8-hydroxy-2-deoxy Guanosine Antibody Diluent	1 vial/13mL
Wash Buffer Concentrate	1 vial/50mL
TMB Substrate	1 vial/ 13mL
Stop Solution	1 vial/ 13mL
Plate Cover	2 covers

Assay Overview

1. Prepare standard and samples in the Sample and Standard Diluent.
2. Add 50 μ L of prepared standards and samples in triplicate to appropriate wells.
3. Add 50 μ L of the diluted antibody preparation to the appropriate wells.
4. Cover plate with Plate Cover and incubate at room temperature (20-25°C) for 1 hour.
5. Wash plate 4 times with 1X Wash Buffer.
6. Add 100 μ L of TMB Substrate to each well.
7. Cover plate and develop the plate in the dark at room temperature for 30 minutes.
8. Add 100 μ L of Stop Solution to each well.
9. Measure absorbance on a plate reader at 450 nm.
10. Plot the standard curve and calculate sample concentrations.

Background

8-hydroxy-2-deoxy Guanosine (8-OH-dG) is produced by the oxidative damage of DNA by reactive oxygen and nitrogen species and serves as an established marker of oxidative stress (1-4). Hydroxylation of guanosine occurs in response to both normal metabolic processes and a variety of environmental factors (i.e., anything that increases reactive oxygen and nitrogen species). Increased levels of 8-OH-dG are associated with the aging process as well as with a number of pathological conditions including cancer, diabetes, and hypertension(5-9). In complex samples such as plasma, cell lysates, and tissues, 8-OH-dG can exist as either the free nucleoside or incorporated in DNA. Once the blood enters the kidney, free 8-OH-dG is readily filtered into the urine, while larger DNA fragments remain in the bloodstream. Because of the complexity of plasma samples, urine is a more suitable matrix for the measurement of free 8-OH-dG than plasma. Urinary levels of 8-OH-dG range between 2.7-13 ng/mg creatine, while plasma levels of free 8-OH-dG have been reported to be between 4-21 pg/ml as determined by LC-MS (10-11).

References

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6. Lee J., et al. (2005) Hypertension 45: 986-990.
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Product Images

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	S1	Blk	Blk	Blk	8	8	8	16	16	16
B	S2	S2	S2	1	1	1	9	9	9	17	17	17
C	S3	S3	S3	2	2	2	10	10	10	18	18	18
D	S4	S4	S4	3	3	3	11	11	11	19	19	19
E	S5	S5	S5	4	4	4	12	12	12	20	20	20
F	S6	S6	S6	5	5	5	13	13	13	21	21	21
G	S7	S7	S7	6	6	6	14	14	14	22	22	22
H	S8	S8	S8	7	7	7	15	15	15	23	23	23

S1 – S7: 60 to 0.94 ng/ml Standards Blk: Blank
 S8: Zero Standard 1 – 23: Samples

Diagram of the Triplicate Sample Plate Format

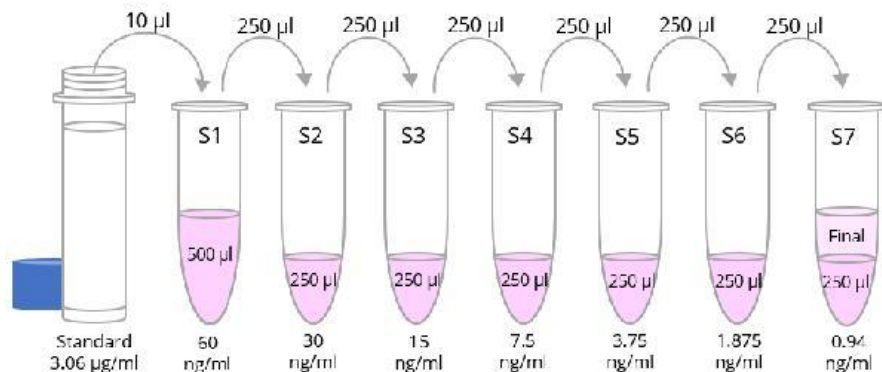
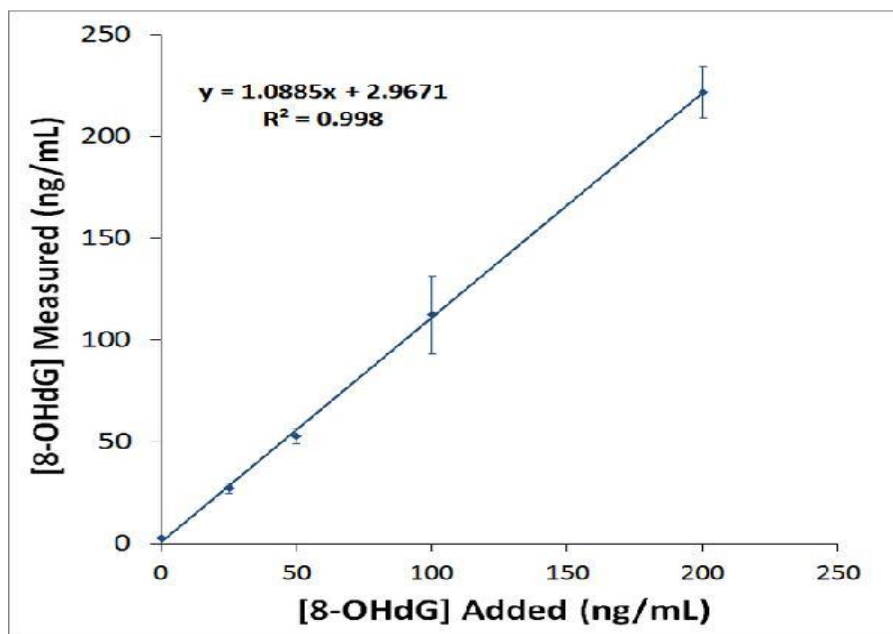


Diagram of the Preparation of the 8-OHdG Standards



Urine Spike Assay

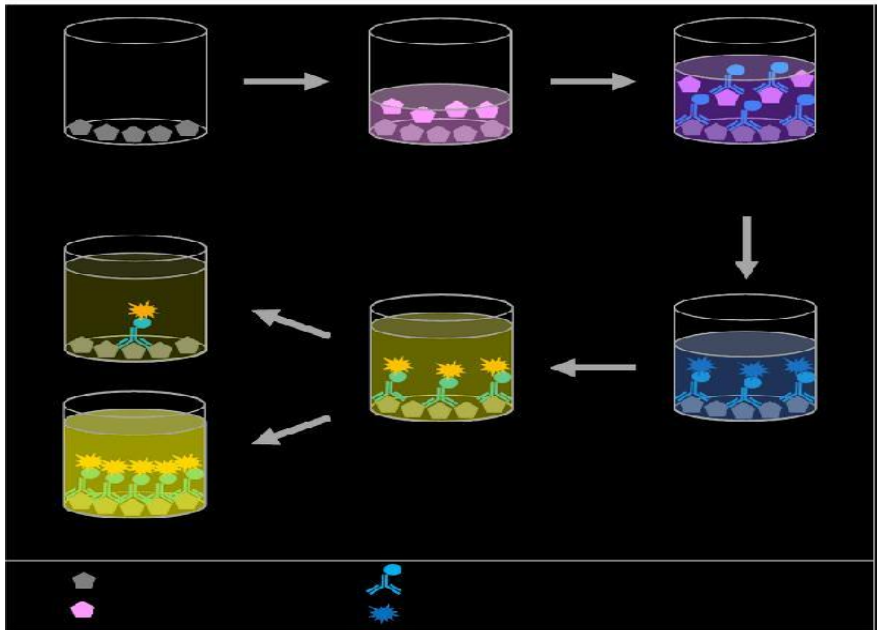
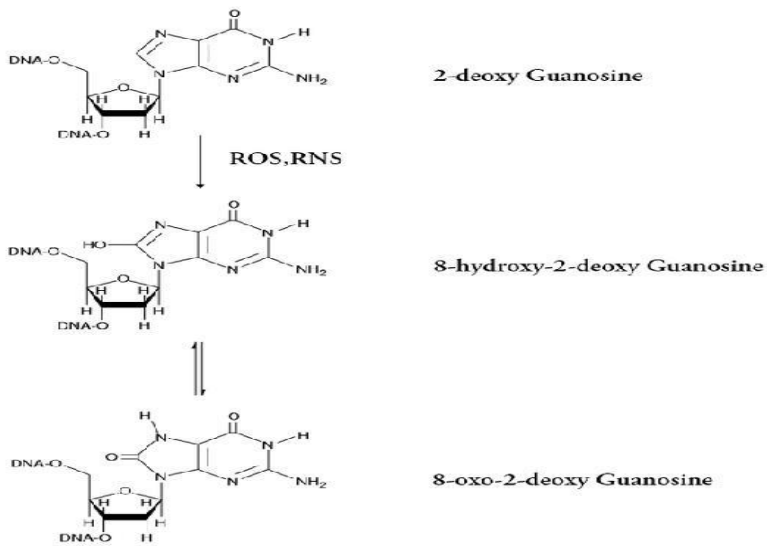
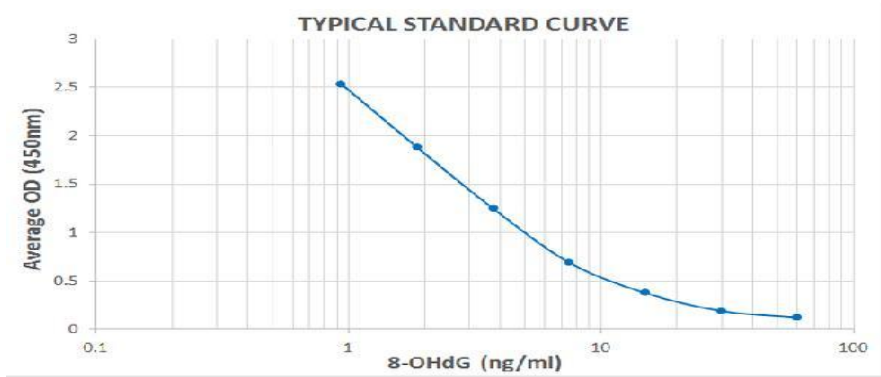


Diagram of the 8-OHdG Competitive ELISA



Chemical Equation of the Oxidation of Guanosine



Typical Standard Curve for the DNA Damage (8-OHdG) ELISA Kit (Enzyme-Linked Immunosorbent Assay)K7114

Assay Type: Competitive ELISA.

Detection Method: Colorimetric Assay.

Assay Range: 0.94 60 ng/ml.