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GENLISA® Human 8-Hydroxydeoxyguanosine, 8-OHDG ELISA

REF: KBH3867

Ver 1.0

PackSize: 96 wells

For Research Use Only

Enzyme Immunoassay for the Quantitative Determination of 8-Hydroxydeoxyguanosine, 8-OHDG in Human serum, plasma and other biological samples

RUO	For Research Use Only	REF	Catalog Number
	Store At	LOT	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Consult Operating Instructions

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REF KBH3867 Σ 96 tests

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Introduction:

The GENLISA® ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs a competitive ELISA technique.

Intended Use:

The GENLISA® Human 8-Hydroxydeoxyguanosine, 8-OHDG ELISA kit is used as an analytical tool for quantitative determination of Human 8-Hydroxydeoxyguanosine, 8-OHDG serum, plasma and other biological samples.

Principle:

The method employs competitive enzyme-linked immunosorbent assay ELISA technique to assay the level of 8-Hydroxydeoxyguanosine, 8-OHDG in samples. Standards or Samples competes with the Human 8-Hydroxydeoxyguanosine, 8-OHDG antigen coated microtiter well, to form a complex with the Biotinylated 8-OHDG antibody. Wells are washed to remove the excess conjugate and Streptavidin:HRP Conjugate is added to the microplate and incubated. After incubation and a washing step TMB Substrate, are added. Blue color develops on incubation and the reaction is stopped with a Stop Solution to form a yellow color. The concentration of the Human 8-Hydroxydeoxyguanosine, 8-OHDG in the samples is inversely proportional to the yellow color developed (absorbance) in the wells.

Materials Provided:

1. Human 8-OHDG Antigen Coated Microtiter Plate (8 x 12 wells) - 1 no
2. Human 8-OHDG Standard (lyophilized, concentrated, 100 ng/ml) - 2 vials
3. Biotinylated 8-OHDG Antibody (lyophilized, concentrated) – 1 vial
4. Streptavidin:HRP Conjugate (concentrated) - 120 ul
5. Sample Diluent 1 - 20 ml
6. Sample Diluent 2 - 20 ml
7. Purified Water – 200 ul
8. Biotin Antibody Dilution Buffer - 10 ml
9. HRP Conjugate Dilution Buffer - 10 ml
10. (20X) Wash Buffer - 25 ml
11. TMB Substrate - 12 ml
12. Stop Solution - 12 ml
13. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Clean tubes and Eppendorf tubes.
6. Precision single and multi-channel pipette and disposable tips.
7. 37°C incubator.
8. Timer.

Handling/Storage:

1. All reagents should be stored as indicated on the component label.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.



Sample Preparation and Storage:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

1. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
2. **Serum-** Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
3. **Plasma-** Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.
4. **Urine-** Collect urine in a sterile container, centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
5. **Saliva -** Collect saliva according to standard procedure in a centrifuge tube. To clarify, centrifuge at 2,000 x g for 15 minutes. Carefully remove supernatant and assay immediately or store at ≤ -20°C in aliquots for later use. Avoid repeated freeze-thaw cycles.
6. **Cell Culture Supernatant-** Collect sample in a sterile container. Centrifuge for 20-mins at 2000-3000 rpm. Remove the supernatant carefully. When examining the components within the cell, dilute cell suspension with PBS (pH 7.2-7.4), if cell concentration is greater than 1 million/ml. Damage the cells by repeated freeze-thaw cycles to release intracellular components. Centrifuge for 20-min at 2000-3000 rpm. If precipitation appears, centrifuge again.
7. **Tissue Samples-** Rinse tissues in PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (pH7.4) with a glass homogenizer on ice. Thaw at 2-8°C or freeze at -20°C. Centrifuge at 2000-3000 RPM for approximately 20 minutes and collect the supernatant carefully.
8. **DNA extraction from other samples-**

a. **Cultured Suspension Cells -** Grow 1-5 x 10^6 cells in suspension using complete medium in a suitable tissue culture plate or flask. Count the cells. Harvest cells by centrifugation and remove growth medium. Wash one time with 1X PBS. Suspend cell pellets at 1x10^6 cells/ml in ice-cold 1X PBS. For example, add 5 mL 1X PBS to 5 x 10^6 cells. Aliquot 1 ml into 1.5 ml microcentrifuge tubes. Centrifuge at 10,000 x g for 10 seconds at 2-8 °C. Discard supernatant. Proceed to DNA Extraction. (Cell pellets can be flash frozen in liquid nitrogen and stored at ≤-70°C for later use.)

b. **Cultured Adherent Cells -** Grow 1-5 x 10^6 adherent cells in complete medium in a suitable tissue culture dish or flask until 75% confluent. Remove the growth medium and harvest cells by trypsinization or a method of choice. Count the cells. Wash one time with 1X PBS. Suspend the cell pellets at 1x10^6 cells/ml in ice-cold 1X PBS. For example, add 5 ml 1X PBS to 5 x 10^6 cells. Aliquot 1 ml into 1.5 ml microcentrifuge tubes. Centrifuge at 10,000xg for 10 seconds at 2-8°C. Discard supernatant. Proceed to DNA Extraction. (Cell pellets can also be flash frozen in liquid nitrogen and stored at ≤ -70°C for later use).

c. **Tissue Samples -** The tissue specimen should be cut into a 2 mm cube and weigh approximately 8-12mg in mass. Proceed to DNA Extraction.

d. **DNA Extraction -** Extract DNA from the above cultured cells or tissue samples by a desired method or commercial extraction kit. (Generally the minimal amount of extracted DNA required for each sample is 20 50µg.) Quantitate DNA spectrophotometrically (OD260 = 50µg/ml). The suggested final DNA concentration is 200µg/ml to 1000µg/ml. Add Mg2+ and Ca2+ to DNA solution (the final concentration of Mg2+ is 2.5-10mM , the final concentration of Ca2+ is 0.5-1mM). Then Add 2µL DNase I (5 Units/µL) per 50µg DNA and incubate for 1 hour at 37°C. DNA is cut to form oligonucleotides and mononucleotides. Then, add 2µL Alkaline Phosphatase (1 Unit/µL) per 50µg DNA and incubate 1 hour at 37°C. Alkaline Phosphatas removes the 5 'end phosphate group of deoxynucleotide to prevent the connection of the 5' end and the 3 'end, so that the deoxynucleotide is in a linearized state, which is conducive to detection. Assay immediately or aliquot and store at ≤ -20°C.

Note: *Grossly hemolyzed samples are not suitable for use in this assay.*

Sample Dilution

The user should estimate the concentration of target protein in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided sample diluent, and several trials may be necessary. The test sample must be well mixed with

the sample diluent. And also standard curves and sample should be making in pre-experiment. If samples with very high concentrations, dilute samples with PBS first and then dilute the samples with the Sample Diluent.

Please refer to the following table of recommended dilution ratio for 8-Hydroxydeoxyguanosine, 8-OHDG samples for reference.

Dilution Fold	Sample	Sample Diluent 1	Sample Diluent 2	Total Diluted Sample Volume
1/2	60 ul	60 ul	---	120 ul
1/5	24 ul	96 ul	---	120 ul
1/10	12 ul	108 ul	---	120 ul
1/20	6 ul	114 ul	---	120 ul
1/50	3 ul	---	47 ul	50 ul + 100 ul Sample Diluent 1
1/100	3 ul	---	177 ul	180 ul + 120 ul Sample Diluent 1
1/1000	2 step dilution. Create a 50 fold dilution and then make a 20 fold dilution Sample diluent 2 is used throughout the dilution.			
1/10000	2 step dilution. Create a 100 fold dilution and then make a 100 fold dilution using Sample diluent 2 is used throughout the dilution.			
1/100000	3 step dilution. Create a 50 fold dilution and then make a 20 fold dilution. Finally create a 100 fold dilution using Sample diluent 2 is used throughout the dilution.			

Note: The volume in each dilution is not less than 3 ul. Dilution factor should be within 100 fold. Mix well during dilution and avoid foaming.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make **Wash Buffer (1X) 500 ml;** dilute **25 ml of (20X) Wash Buffer in 475 ml of DI water**.
4. **Biotinylated 8-OHDG Antibody Working Solution**: Prepare it within 30 minutes before experiment.
   Dissolve: Centrifuge for 1min at 2000xg and bring down the concentrated biotin-labeled antibody to the bottom of tube. Add 70ul purified water into tube and mix them thoroughly, after the biotin-labeled antibody is dissolved; please store it at 2-8°C.
   Calculate required total volume of the working solution: 0.05 ml / well x quantity of wells. (Allow 0.1-0.2 ml more than the total volume. Dilute the Biotinylated 8-OHDG Antibody (concentrated) with Biotin Antibody Dilution Buffer at 1:100 and mix them thoroughly (i.e. Add 1 ul Biotinylated 8-OHDG Antibody into 99 ul Biotin Antibody Dilution Buffer).
5. **Streptavidin:HRP Conjugate Working Solution**: Prepare it within 30 minutes before experiment. Calculate required total volume of the working solution: 0.1ml / well x quantity of wells. (Allow 0.1-0.2 ml more than the total volume. Dilute the Streptavidin:HRP Conjugate with Streptavidin:HRP Conjugate Dilution Buffer at 1:100 and mix them thoroughly (i.e. Add 1 ul of Streptavidin:HRP Conjugate into 99 ul of Streptavidin:HRP Conjugate Dilution Buffer).
6. **Standards Preparation**: Reconstitute original 8-Hydroxydeoxyguanosine, 8-OHDG Standard with 1.0 ml of Sample Diluent 1. Keep the standard for 10 mins with gentle agitation before making further dilutions. Prepare the additional Standards by serially diluting the standard stock solution as per the below table.

Standard Concentration	Standard Vial	Dilution Particulars
100 ng/ml	Standard No.8	Reconstitute with 1 ml Sample Diluent 1
50 ng/ml	Standard No.7	300 ul Standard No.8 + 300 ul Sample Diluent 1
25 ng/ml	Standard No.6	300 ul Standard No.7 + 300 ul Sample Diluent 1
12.5 ng/ml	Standard No.5	300 ul Standard No.6 + 300 ul Sample Diluent 1
6.25 ng/ml	Standard No.4	300 ul Standard No.5 + 300 ul Sample Diluent 1
3.125 ng/ml	Standard No.3	300 ul Standard No.4 + 300 ul Sample Diluent 1
1.562 ng/ml	Standard No.2	300 ul Standard No.3 + 300 ul Sample Diluent 1
0 ng/ml	Standard No.1	300 ul Sample Diluent 1 only

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.

2. High Dose Hook Effect may be observed in samples with very high concentrations of 8-Hydroxydeoxyguanosine, 8-OHDG. High Dose Hook Effect is due to excess of antibody for very high concentrations of Human 8-Hydroxydeoxyguanosine, 8-OHDG present in the sample.
3. Human 8-Hydroxydeoxyguanosine, 8-OHDG concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
4. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Human 8-Hydroxydeoxyguanosine, 8-OHDG.
5. It is recommended that all Standards and Samples be assayed in duplicates or triplicates.
6. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
7. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
8. The plates should be read within 30 minutes after adding the Stop Solution.
9. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay.

2. Add **50 ul prepared Standards and Samples** to respective wells.

3. Pipette **50 ul Biotinylated 8-OHDG Antibody Working Solution** to all wells.

4. Cover the plate with a sealer and incubate for 45 minutes at 37°C.

5. Aspirate and wash plate 4 times with diluted Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

6. Pipette **100 ul Streptavidin:HRP Conjugate Working Solution** to all wells. Mix well.

7. Cover the plate with a sealer and incubate for 30 minutes at 37°C.

8. Aspirate and wash as per Step (5) above.

9. Pipette **100 ul TMB Substrate** in all the wells.

10. Incubate the plate at **37°C** for **10 minutes**. DO NOT SHAKE or else it may result in higher backgrounds and worse precision.

11. Pipette **100 ul** of **Stop Solution** to all wells. The wells should turn from blue to yellow in color.

12. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Calculation of Results:
Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Graph Paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Human 8-Hydroxydeoxyguanosine, 8-OHDG concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Human 8-Hydroxydeoxyguanosine, 8-OHDG Concentration.

If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit or 4-PL is best recommended for automated results.

Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.

Quality Control:
It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Performance Characteristics of the Kit:
This kit has been validated. Please view the details herein below.

Standard Calibration Range:
1.562 ng/ml – 100 ng/ml.

Sensitivity:
Limit of Quantification: It is defined as the lowest detectable concentration that can be determined with an acceptable repeatability and the LOQ was found to be 0.938 ng/ml.

Specificity:
This assay has high sensitivity and excellent specificity for detection of 8-Hydroxydeoxyguanosine, 8-OHDG. No significant cross-reactivity or interference between 8-Hydroxydeoxyguanosine, 8-OHDG and analogues was observed.

Recovery
Matrices listed below were spiked with certain level of 8-Hydroxydeoxyguanosine, 8-OHDG and the recovery rates were calculated by comparing the measured value to the expected amount of 8-Hydroxydeoxyguanosine, 8-OHDG in samples.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	85-105	96
EDTA Plasma(n=5)	85-105	95
Heparin Plasma(n=5)	85-100	97

Precision:
Intra-Assay: CV<8%
Inter-Assay: CV<10%

Linearity
The linearity of the kit was assayed by testing samples spiked with appropriate concentration of 8-Hydroxydeoxyguanosine, 8-OHDG and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation.

Sample	1:2	1:4	1:8
Serum(n=5)	85-100%	80-95%	88-105%
EDTA Plasma(n=5)	82-98%	84-101%	90-98%
Heparin Plasma(n=5)	80-96%	81-99%	81-100%

Safety Precautions:

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Source materials maybe derived from Human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed.
- Do not smoke, eat or drink while handling kit material.
- Always use protective gloves.
- Never pipette material by mouth.
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

- In any case GLP should be applied with all general and individual regulations to the use of this kit.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	Interpolated Concentration
1A 2A	Standard No.1 Standard No.1			
1B 2B	Standard No.2 Standard No.2			
1C 2C	Standard No.3 Standard No.3			
1D 2D	Standard No.4 Standard No.4			
1E 2E	Standard No.5 Standard No.5			
1F 2F	Standard No.6 Standard No.6			
1G 2G	Standard No.7 Standard No.7			
1H 2H	Standard No.8 Standard No.8			
3A 4A	Sample Sample			
3B 4B	Sample Sample			

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SYMBOLS KEY

MTP	Coated Microtiter Plate (8x12 wells)
STD	Standard
BIOTIN AB	Biotinylated Antibody
HRP CONJ	Conjugate Horseradish Peroxidase
BIOTIN DIL	Biotin Antibody Dilution Buffer
HRP DIL	HRP Conjugate Dilution Buffer
SAMP DIL 1	Sample Diluent 1
SAMP DIL 2	Sample Diluent 2
PURE WATER	Purified Water
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature