8 Hydroxyguanosine (8-OHdG) ELISA Kit

Enzyme Immunoassay for the quantitative determination of 8-OH-dG in urine, cell culture supernatant, serum, plasma, saliva and cell/tissue lysate.

Catalog number: ARG80911

Package: 96 wells

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INTRODUCTION

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. 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. 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.

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. A highly purified 8-hydroxy-2-deoxy Guanosine (8-OH-dG) has been pre-coated onto a microtiter plate. 8-OH-dG in samples and the 8-OH-dG coated on wells are competing for a limited amount of HRP-conjugated 8-OH-dG monoclonal antibody.

After incubation, the wells are washed with wash buffer to remove unbound material. The substrate solution (TMB substrate) is added and incubated,

stopped with STOP solution, resulting in the development of a distinct color which absorbance is measured at 450 nm. The concentration of 8-OH-dG in samples or standards is indirectly proportional to the color intensity or OD readings by the spectrometer.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received aliquot standard stock into smaller portions and then stored at -20°C, store the other components at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
8-OH-dG/BSA coated microplate	96 wells	-20°C
8-OH-dG standard (3.06 µg/ml)	1 vial (100 µl)	-20°C
8-OH-dG HRP-conjugated antibody	1 vial (75 µl)	-20°C
Sample/Standard Diluent	50 ml (Ready to use)	4°C
8-OH-dG antibody Diluent	13 ml (Ready to use)	4°C
10X Wash Buffer Concentrate	50 ml	4°C
TMB substrate	13 ml (Ready to use)	4°C, keep in dark
STOP solution	13 ml (Ready to use)	4°C
Plate Cover	2	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store standard stock at -20°C and store the other components in the kit at 4°C at all times.
- The Stock Standard should be aliquoted into smaller portions before use to ensure product integrity and store the aliquoted Stock Standard at -20°C. Avoid repeated freeze-thaw cycles
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Avoid microbial contamination of reagents and equipment. Automated plate washers can easily become contaminated thereby causing assay variability.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB

solution to glass, foil or metal. If the solution is blue before use, do NOT use it.

- Change pipette tips between the addition of different reagent or samples.
- Use a new adhesive plate cover for each incubation step.
- Always add the Antibody Preparation after the rest of the reagents, as this is a competitive assay.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated. Prepare at least 180µL of your diluted sample to permit assay in triplicate.

Note:

- All samples must be free of organic solvents prior to assay.

- Samples that cannot be assayed immediately should be stored as indicated below.

- Please be advised that all suggested dilutions below are simply recommended as a starting point, and it may be necessary to adjust the

dilution based on experimental results.

Urine – Urine samples should be centrifuged at 2,000 X g for 10 minutes, or filtered with 0.2 µm filter, and stored at-20°C immediately after collection. Dilute urine samples **1:20** in sample/standard diluent as starting dilution prior to testing. For example: 9 µL of sample into 171 µL of Standard/Sample Diluent. Urinary concentrations of 8-OHdG can vary considerably and can be standardized against creatinine levels if required.

Saliva – Saliva samples should be stored at-80°C immediately after collection. Samples may be assayed directly after appropriate dilution. Dilute saliva samples **1:8** in sample/standard diluent as starting dilution prior to testing.

Cell Culture Supernatants – Collect culture media samples and store at -80°C. Fetal Bovine Serum contains 8-OH-dG, therefore assays should either be performed in serum-free medium or PBS. These samples may be assayed directly. If the 8-OHdG concentration is high enough to dilute the sample 10-fold with Sample/Standard Diluent, the assay can be performed without any modifications. When assaying less concentrated samples (where samples cannot be diluted 1:10 with Sample/Standard Diluent), dilute the standards in the same culture medium as that used for the experiment. This will ensure that the matrix for the standards is comparable to the samples. We recommend that a standard curve be run first to ensure that the assay will perform in a particular culture medium.

Serum / Plasma - The concentration of free 8-OHdG in plasma is very low relative to the level of DNA incorporated 8-OHdG. Glomerular filtration results in excretion of 8-OHdG into the urine, while the DNA-incorporated 8-OHdG remains in the blood. The differing fates of free versus DNA-incorporated 8-

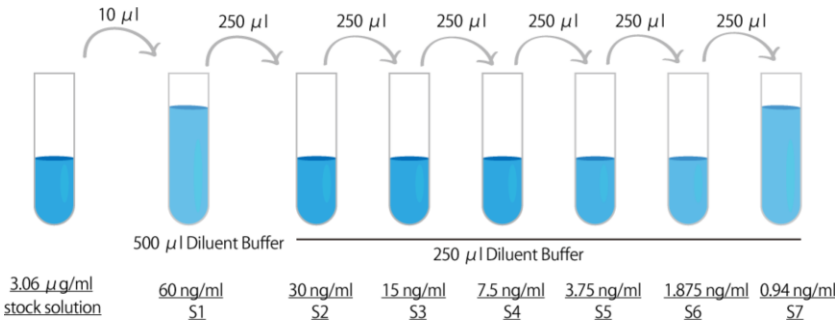
OHdG should be considered in experimental design. If you choose to measure DNA-incorporated 8-OHdG in plasma, it is possible to purify DNA using a commercially available kit and treat the DNA with a combination of nuclease and alkaline phosphatase to liberate the individual bases. Due to the complexities of measuring 8-OHdG in plasma, urine is often a more appropriate matrix. Collect serum/plasma and assay immediately or aliquot and store samples at -80°C. Avoid repeated freeze-thaw cycles. Serum samples may be diluted 1:20 (v:v) in Sample/Standard Diluent as the starting dilution prior to testing.

Cell Lysates - Collect lysates using established methods and store at -80 °C. Purify DNA using a commercially available extraction kit. Digest DNA using nucleus P1 (For example: Sigma N8630 or equivalent) as according to manufacturer's instructions. Adjust pH to 7.5-8.5 using 1M Tris. Add 1 unit of Alkaline Phosphatase per 100µg of DNA and incubate at 37°C for 30 minutes. Boil for 10 minutes and place on ice until use.

Tissue samples – Snap-freeze tissue samples in liquid Nitrogen immediately after collection. Store at -80°C until use. When ready to use the samples, thaw and add 5 ml homogenization buffer (0.1M PBS, pH7.4 containing 1 mM EDTA) per gram of tissue. Homogenize the sample using either a Polytron-type homogenizer or a sonicator. Centrifuge at 1000xg for 10 minutes and purify supernatant using a commercially available DNA extraction kit. Digest DNA using nucleus P1 (For example: Sigma N8630 or equivalent) as according to manufacturer's instructions. Adjust pH to 7.5-8.5 using 1M Tris. Add 1 unit of Alkaline Phosphatase per 100µg of DNA and incubate at 37 °C for 30 minutes. Boil for 10 minutes and place on ice until use.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 50ml of 10X Wash buffer into a total of 500 ml with distilled water. Store 1X Wash buffer at 2-8°C for up to 1 month. Do not use 1X Wash Buffer if it becomes visibly contaminated during storage.
- 8-OH-dG HRP-conjugated antibody: Determine the amount of antibody required. For every strip –well used (8 wells), prepare 0. 5ml of antibody solution. To prepare antibody working solution, dilute the HRP-conjugated 8-OH-dG Antibody 1:100 with antibody diluent. For example, if 6 ml of antibody working solution is needed, dilute 60 µl of stock antibody into 6 ml antibody diluent. Mix well prior to use.
- Standards: Centrifuge standard vial before removing the cap. Add 10 µl of concentrated stock standard solution into 500 µl of Standard/Sample Diluent buffer and mix well to make a 60 ng/ml Standard S1. The Sample/Standard Diluent buffer serves as zero standard (S0, 0 ng/ml), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: S1: 60 ng/ml, S2: 30 ng/ml, S3: 15 ng/ml, S4: 7.5 ng/ml, S5: 3.75 ng/ml, S6: 1.875 ng/ml, S7: 0.94 ng/ml. *The Stock Standard should be aliquoted into smaller portions before use to ensure product integrity and store the aliquoted Stock Standard at -20°C. Avoid repeated freeze-thaw cycles.*



Dilute 8-OH-dG standard as according to the table below:

Standard	8-OH-dG Conc. (ng/ml)	µl of Sample/Standard Diluent	µl of standard
S1	60 ng/ml	500	10 (3.06 µg/ml Stock)
S2	30 ng/ml	250	250 (S7)
S3	15 ng/ml	250	250 (S6)
S4	7.5 ng/ml	250	250 (S5)
S5	3.75 ng/ml	250	250 (S4)
S6	1.875 ng/ml	250	250 (S3)
S7	0.94 ng/ml	250	250 (S2)
S0	0	250	0

Note: For Cell Culture Supernatants samples, if the concentrations of 8-OH-dG maybe low, the standards have to be diluted by the same culture medium. Please refer the sample preparation section for the detail.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards, samples and controls should be assayed in at least duplicates (triplicate is recommended).

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **50 µl** of **standards (S0-S7) or samples** in duplicate into appropriate wells.
3. Add **50 µl** of **8-OH-dG HRP-conjugated antibody working solution** into each wells except for Blank wells.
4. Add **50 µl** of **standard/sample diluent** and **50 µl** of **antibody diluent** into another wells as Blank wells.
5. Cover plate with Plate Cover and incubate at **room temperature (20-25°C) for 1 hour**.
6. Carefully remove adhesive plate cover. Gently squeeze the long sides of the plate frame before washing to ensure all strips remain securely in the frame.
7. Aspirate each well and wash, repeating the process 3 times for a **total 4 washes.** Wash by filling each well with **1X Wash buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
8. Add **100 µl** of **TMB substrate** into each well. Cover and incubate for **30**

minutes at RT in dark. The substrate reaction yields a blue solution.

9. Add 100 µl of STOP solution into each well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough mixing.
10. Wipe underside of wells with a lint-free tissue. Read the OD with a microplate reader at 450 nm immediately or within 30 min after adding STOP solution.

CALCULATION OF RESULTS

1. Calculate the average Net Optical Density (OD) bound for each standard and sample by subtracting the average Blank OD from the average OD bound.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Calculate the average Net Optical Density (OD) bound for each standard and sample by subtracting the average Blank OD from the average OD bound.

4. Plot Net OD versus Concentration of 8-OHdG for the standards. Sample concentrations may be calculated off of Net OD values using the desired curve fitting.

5. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

6. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give

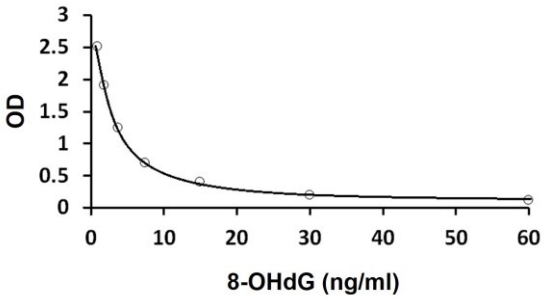
slightly different results.

7. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

8. Samples that read at concentrations outside of the standard curve range will need to be re-analyzed using a different dilution. Make sure to multiply sample concentrations calculated off the curve by the dilution factor used during sample preparation to get starting sample concentration.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of 8-OH-dG ranged from 0.94-60 ng/ml.

The mean MDD was 0.59 ng/ml.

Intra and inter-assay precision

% CV of Intra assay precision: < 10%.

% CV of Inter assay precision: < 10%.

Specificity

The cross-reactivity was found with the following factors:

8-hydroxy-2-deoxy Guanosine: 100%

8-hydroxy Guanosine: 23%

8-hydroxy Guanine: 23%

Guanosine: <0.01%