Signosis, Inc.
Innovative Plate Assay Solutions

8-OHdG DNA Damage ELISA Kit for Cells/Tissue

Catalog # EA-7085-B **(For Research Use Only)**

Introduction

8-Hydroxy-2-deoxyguanosine (8-OHdG) is a key biomarker of oxidative DNA damage and an important indicator of cellular oxidative stress. It forms when reactive oxygen species attack the guanine bases within DNA, leading to structural modifications that can disrupt genetic stability. Elevated levels of this molecule reflect increased oxidative burden and impaired antioxidant defenses. Understanding and monitoring 8-OHdG is therefore essential for advancing research into disease mechanisms, evaluating antioxidant therapies, and developing strategies to promote long-term cellular health.

Principle

In cells and tissues, 8-OHdG is incorporated in the DNA as a nucleotide. Because of this, DNA extraction and digestion is necessary to break down the DNA-incorporated 8-OHdG into smaller fragments so the 8-OHdG can be detected.

In red and white blood cells, 8-OHdG is also present in its DNA-incorporated form and needs to be extracted before it can be measured.

Competitive ELISA

The 8-OHdG assay uses the competitive ELISA format, which is ideal for small-molecule biomarkers lacking multiple epitopes for sandwich detection. Plates are pre-coated with 8-OHdG-protein conjugate that competes with free 8-OHdG in samples for binding to a specific anti-8-OHdG rabbit antibody. After washing away unbound material, HRP reagent binds to the antibodies bound to the plate. TMB substrate generates a blue color proportional to bound antibody, turning yellow upon acidification. Absorbance at 450 nm is inversely proportional to 8-OHdG concentration in the sample—higher free 8-OHdG levels block the antibody from binding to the plate.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm.
2. Adjustable pipettes and a repeat pipettor.
3. Deionized or distilled water

Assay Precautions

1. All ELISA reagents must be at room temperature before use.
2. Vigorous plate washing is essential.
3. Use new disposable pipette tips for each transfer to avoid cross-contamination.
4. Use a new adhesive plate cover for each incubation step.
5. Minimize lag time between wash steps to ensure the plate does not become completely
6. dry during the assay.
7. Avoid microbial contamination of reagents and equipment. Automated plate washers
8. can easily become contaminated thereby causing assay variability.
9. Take care not to contaminate the TMB Substrate. Do not expose the TMB solution
10. to glass, foil or metal. If the solution is blue before use, do NOT use it.
11. Individual components may contain preservatives. Wear gloves while preforming the assay. Please follow proper disposal procedures.
12. Include a standard curve each time the assay is performed.
13. Run both standards and samples in triplicate.
14. Buffers may crystallize over time. Warm crystallized buffer until the salt crystals return
15. to solution. Ensure that your components return to RT before use in the assay

****Spin down small tubes before starting experiment. ****

Sample Preparation

Cell Lysate Preparation

Purify the sample with a commercially available DNA extraction kit, then digest the DNA using nuclease P1 (Sigma N8630 or a comparable enzyme) according to the supplier's instructions. Adjust the pH to between 7.5 and 8.5 using 1 M Tris buffer. Add alkaline phosphatase at 1 unit per 100 µg of DNA and incubate at 37 °C for 30 minutes. After incubation, boil the sample for 10 minutes and place it on ice until needed. Use 300 ng–3 µg total DNA input. The DNA stock concentration should be approximately 1–5 µg/µL, and the appropriate volume of DNA is added to the reaction

Tissue Sample Preparation

When the samples are ready for use, thaw them and add 5 mL of homogenization buffer (0.1 M phosphate buffer, pH 7.4, supplemented with 1 mM EDTA) per gram of tissue. Homogenize the tissue using a Polytron homogenizer or sonication. Centrifuge the homogenate at 1,000 × g for 10 minutes, then collect the supernatant and purify the DNA using a commercially available extraction kit. Digest the purified DNA with nuclease P1 (Sigma N8630 or a similar enzyme) according to the manufacturer's instructions. Adjust the pH to 7.5–8.5 with 1 M Tris buffer, then add alkaline phosphatase at a ratio of 1 unit per 100 µg of DNA and incubate at 37 °C for 30 minutes. Finally, boil the sample for 10 minutes and cool it on ice until further use. Use 300 ng–3 µg total DNA input. The DNA stock concentration should be approximately 1–5 µg/µL, and the appropriate volume of DNA is added to the reaction

Culture Media Sample Preparation

Fetal bovine serum contains measurable levels of 8-OHdG, so assays should be carried out in serum-free medium or PBS; such samples can be analyzed directly. If the 8-OHdG levels are sufficiently high to allow a 10-fold dilution with Sample and Standard Diluent, the assay can proceed without modification. For samples with lower concentrations that cannot be diluted 1:10, prepare the standards in the same culture medium used for the samples to ensure comparable matrix conditions. It is recommended to first generate a standard curve to confirm that the assay performs reliably in the chosen culture medium. Use 300 ng–3 µg total DNA input. The DNA stock concentration should be approximately 1–5 µg/µL, and the appropriate volume of DNA is added to the reaction

Plasma Sample Preparation

The level of free 8-OHdG in plasma is much lower than that of DNA-bound 8-OHdG. If you choose to measure DNA-incorporated 8-OHdG in plasma, it may be preferable 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. Use 300 ng–3 µg total DNA input. The DNA stock concentration should be approximately 1–5 µg/µL, and the appropriate volume of DNA is added to the reaction

Assay Procedure

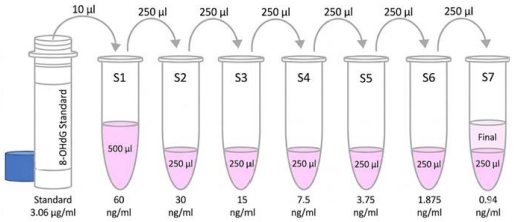
<u>Reagent preparation before starting experiment:</u>

- *Prepare 1X Wash buffer by diluting 10X Wash Buffer in distilled or deionized water*. For example, if preparing 500mL of 1X Wash Buffer, dilute 50 mL of 10X Wash Buffer into 450 mL of distilled water. Mix well. Store reconstituted 1X Wash Buffer at 2-8ºC for up to one (1) month. Do not use 1X Wash Buffer if it becomes visibly contaminated during storage.
- *Antibody Preparation:* Determine the amount of Antibody Preparation required.
  Prepare Antibody Preparation by diluting the 8-hydroxy-2-deoxy Guanosine: HRP Conjugate Antibody Concentrate 1:200 with 8-hydroxy-2-deoxy Guanosine Antibody Diluent.

1. Prepare standard and samples in the Sample and Standard Diluent.

Standard Preparation (S1-S8)
NOTE: The Standard should be aliquoted into smaller portions before use to ensure product integrity. Avoid freeze/thaw cycles. (10 µL of Standard can prepare a triplicate standard curve).
1. Centrifuge the 8-hydroxy-2-deoxy Guanosine Standard vial before removing the cap. This process will ensure that all of the standard is collected and available for use.
2. Label seven (7) polypropylene tubes, each with one of the following standard values: 60 ng/mL, 30 ng/mL, 15 ng/mL, 7.5 ng/mL, 3.75 ng/mL, 1.875 ng/mL and 0.94 ng/mL.
3. Add 500 µL of Sample and Standard Diluent to Tube #1.
4. Add 250 µL of Sample and Standard Diluent to Tube #2, 3, 4, 5, 6 and 7.
5. Add 10 µL of the 3.06 µg/mL 8-hydroxy-2-deoxy Guanosine Standard to Tube #1 for a concentration of 60 ng/mL. Mix well.
6. Transfer 250 µL from Tube #1 to Tube #2. Mix well.

7. Similarly, complete the dilution series to generate the remaining standards (250 µL from Tube #2 to Tube #3, mix well, etc.) up to and including Tube #7.
8. Finally, add 250 µL Sample and Standard Diluent to another 1.5mL polypropylene tube (Tube #8), which is the zero standard (0 ng/mL).

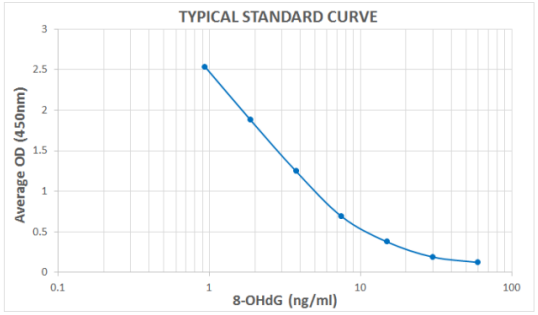


2. Add 50 µL of prepared standards and samples in triplicate to appropriate wells.
3. Add 50 µL of the diluted antibody preparation to the appropriate wells.

Well	Standard OR Sample Preparation	Standard and Sample Diluent	Antibody Preparation	Antibody Diluent	Total Volume Per Well
Standard (S1-S7)	50 µL	Included in Standard Prep	50 µL	Included in Ab. Prep	100 µL
Zero Standard (S8)	-	50 µL	50 µL	Included in Ab. Prep	100 µL
Blank	-	50 µL	-	50 µL	100 µL
Samples	50 µL	Included in Sample Prep	50 µL	Included in Ab. Prep	100 µL

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 µL of TMB Substrate to each well. Notes: *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.*
7. Cover plate and develop the plate in the dark at room temperature for 30 minutes.
8. Add 100 µL of Stop Solution to each well. *Notes: Only remove the required amount of TMB Substrate and Stop Solution for the number of strips being used. Do NOT use a glass pipette to measure the TMB Substrate solution. Do NOT return leftover TMB Substrate to bottle. Tap plate gently to mix. The solution in the wells should change from blue to yellow.*
9. Measure absorbance on a plate reader at 450 nm. *Note: Evaluate the plate within 30 minutes of stopping the reaction*
10. Plot the standard curve and calculate sample concentrations

8-OHdG Standard Curve



NOTE: This typical standard curve was generated using the 8-OHdG ELISA Kit Protocol. This standard curve is for demonstration only. A standard curve must be generated for each assay. Assay Range: 0.94-60 ng/mL