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 Required but Not Provided

- Microplate reader capable of measuring absorbance at 450nm.
- DNA Damage Extraction Kit (EA-7089)

Materials Provided

- 8-OHdG ELISA Plate (4°C)
- Dilution Buffer (4°C)
- 5x Assay Wash Buffer (4°C)
- 8-OHdG Standard (-20°C)
- Detection Antibody (-20°C)
- HRP Reagent (4°C)
- Substrate (4°C)
- Stop Solution (4°C)

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

Cell and Tissue DNA Extraction (Recommended)

1. For higher-quality and optimal results, we recommend using the DNA Damage Extraction Kit (EA-7089) to prepare your cell or tissue samples for 8-OHdG analysis.

Cell Lysate Preparation (Direct Method)

1. Wash the cells once with PBS before lysing the cells.
2. For a 96-well culture plate, add 40 µL of lysis buffer to each well and incubate at room temperature for 10 minutes.
3. Pipette the lysis buffer up and down to detach the cells and transfer the cell lysates into a new tube.
4. If necessary, homogenize the cell lysates with a sonicator.
5. The cell lysates may be assayed directly or stored at -80°C.
6. Use PBS to dilute the tissue sample to the appropriate concentration, if necessary.

Tissue Sample Preparation (Direct Method)

1. Weigh tissue sample and add 1 mL of lysis buffer per 100mg of tissue.
2. Homogenize the tissue samples with a tissue grinder.
3. If necessary, further homogenize the tissue samples with a sonicator.
4. Centrifuge the sample at 10,000 RPM for 5 minutes to pellet the tissue debris.
5. Collect the supernatant and measure the protein concentration of the supernatant. The tissue sample can be assayed directly or stored at -80°C.
6. Use PBS to dilute the tissue sample to the appropriate concentration, if necessary.

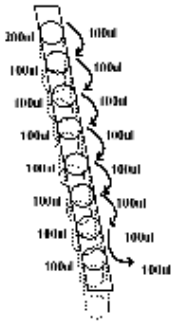
Assay Procedure

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  - 40 ml 5x Assay wash buffer
  - 160 ml ddH2O.
- Dilute Detection antibody 50 times with 1x Diluent buffer.
- Dilute HRP reagent 200 times with 1x Diluent buffer.

1. Standard curve preparation: Add 200 µL of 1x Diluent buffer to the first well of a strip and add 100 µL of 1x Diluent buffer to wells 2–8. Prepare a 100 ng/mL 8-OHdG standard in the first well by mixing 2 µL of the provided 10 mg/mL 8-OHdG standard with the 200 µL 1x Diluent buffer in the first well. Then, serially dilute 100 µL across wells 2–7 as shown in the diagram below. Make sure to discard 100 µL from the seventh, final well of the serial dilution. Leave the eight well untouched as a blank control.

Standard#	8-OHdG Concentration (ng/mL)
1	100
2	50
3	25
4	12.5
5	6.25
6	3.125
7	1.5625
8	0



2. Add 100 µL of sample to each well of the ELISA plate and incubate for 1 hour at room temperature with gentle shaking.
3. Aspirate each well and wash by adding 200 µL of 1x Assay wash buffer. Repeat the process three times for a total of three washes. Make sure all liquid is removed after each wash.
4. Add 100 µL of diluted Detection antibody to each well and incubate for 1 hour at room temperature with gentle shaking.
5. Repeat the aspiration/wash step in step 3.
6. Add 100 µL of diluted HRP reagent to each well and incubate for 45 minutes at room temperature with gentle shaking.
7. Repeat the aspiration/wash step in step 3.
8. Add 100 µL of Substrate to each well and incubate for 10-30 minutes. **Substrate incubation time may vary due to differences in antibody reactivity. Stronger blue signals can be stopped earlier. Weaker signals can be incubated for a longer time up to 1 hour.**
9. Add 50 µL of Stop solution to each well. The color in the wells should change from blue to yellow.
10. Immediately measure the absorbance of the plate at 450 nm using a plate reader.

8-OHdG Standard Curve

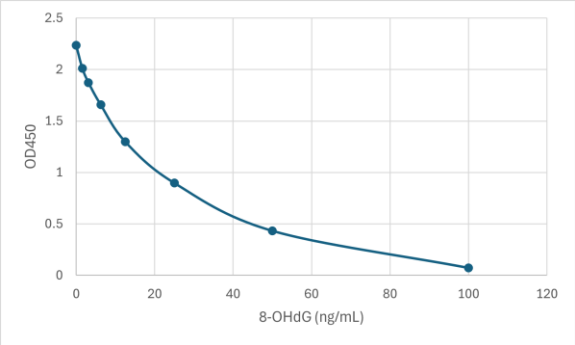


Figure 1. A 100 ng/mL 8-OHdG standard was serially diluted two-fold on an 8-OHdG–coated ELISA plate and quantified using a competitive ELISA with an anti–8-OHdG antibody.