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## 8-OHdG DNA Damage ELISA Kit for Urine/Media

Catalog # EA-7085-A

(For Research Use Only)

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### 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

Free 8-OHdG forms when it is excised from DNA by repair enzymes. Because free 8-OHdG is excreted and concentrated in urine, urine samples are the most ideal medium for measuring 8-OHdG. Free 8-OHdG is also excreted by cultured cells into their surrounding media and can be detected in cell culture supernatant.

Free 8-OHdG is also present in plasma and serum, but at very low concentrations and may require high measurement sensitivity to be detected.

### 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.

### 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. \*\***

**Urine Sample Preparation**

1. Urine samples can be assayed directly or stored away at -80°C.

**Cell Culture Medium Preparation**

1. Cell culture medium can be assayed directly or stored away at -80°C.

**Plasma/Serum Preparation**

1. Centrifuge citrated or EDTA-collected blood at 4°C (1,000 x g for 10 minutes) to separate plasma from erythrocytes. Alternatively, blood collected without anticoagulant can be centrifuged to collect serum.
2. Transfer the plasma layer to a new tube without disturbing the buffy layer.
3. The plasma may be assayed directly or stored away at -80°C.

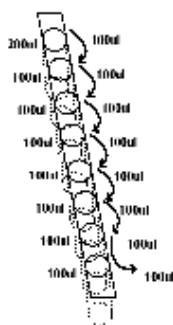
## Assay Procedure

### Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  - 40 ml 5x Assay wash buffer
  - 160 ml ddH<sub>2</sub>O.
- Dilute Detection antibody 50 times with 1x Diluent buffer.
- Dilute HRP reagent 200 times with 1x Diluent buffer.

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

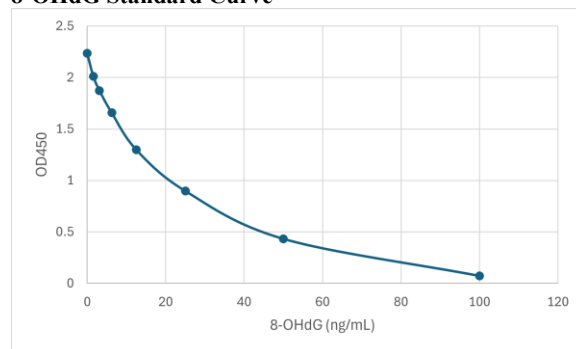


Figure 1. A 100 ng/mL 8-OHdG standard was serially diluted two-fold on an 8-OHdG-coated ELISA plate and quantified using a competitive ELISA with an anti-8-OHdG antibody.