Bowei-biotech
for better bioassays

8-OHdG ELISA

For Research Use Only
Cat. No.: BW-8-OHdG ELISA

1. Introduction

Reactive oxygen species play an important role in different kinds of DNA damages, which is known to contribute to age-related degenerative processes and diseases. Among the identified oxidative DNA damage products, 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-OHdG) is one of the predominant forms of oxidative DNA lesion. 8-OHdG is produced by hydroxyl radical attack at the C-8 position of DNA guanine followed by a one-electron oxidation. Determination of 8-oxodG level has been cited as a preferential non-invasive biomarker of DNA damage induced by oxidative stress, and used to determine the overall effects of oxidative stress, to assess the risk, diagnose, and evaluate the treatment of autoimmune, inflammatory, neurodegenerative and cardiovascular diseases, diabetes, cancer and other age-related diseases [1, 2, 3].

This kit is used for sensitive and specific determination of 8-OHdG levels in biological samples.

2. Principle of the Assay

The principle of the 8-OHdG ELISA follows the typical competitive immunoassay principle.

Competition reaction occurs between the 8-OHdG coated on microwell surface and the 8-OHdG present in calibrators, controls and samples for binding a limited number of anti-8OHdG monoclonal antibody. The washing procedures remove unbound antibodies. After the washing, a sheep anti-mouse antibody-HRP conjugate is added for tracing the bound anti-8OHdG monoclonal antibody. After another washing, the bound enzyme conjugate is detected by the addition of a substrate that generates color by HRP. The intensity of the color formed is inversely proportional to the concentration of 8-OHdG in the sample. A set of calibrators is used to plot a calibrator curve from which the concentration of 8-OHdG in samples and controls can be directly read.

3. Package Components

Each 8-OHdG ELISA package contains reagents for 96 assays.
The expiry date of the complete package is stated on the outer label.

Components	Specification	Description
① Reaction Plate	8×12 wells, detachable	8-OHdG coated plate
② Primary Antibody	0.5ml, 25×	Monoclonal antibody
③ Assay Buffer	30ml	Phosphate Buffered Saline with BSA and Tween-20
④ Secondary Antibody	0.5ml, 25×	HRP-labeled sheep anti-mouse antibody
⑤ Chromogenic Substrate	15ml	TMB substrate used for color development
⑥ Wash Buffer	25ml, 10×	0.05% Tween-20/Phosphate Buffered Saline
⑦Reaction Stop Solution	15ml	1M Citric acid solution
⑧8-OHdG calibrators	6 vials (1ml each)	0, 0.2, 0.5, 1.25, 2.5, 5ng/ml

4. Storage and Shelf Life

- Store in a refrigerator (2~8℃). Desiccant bag must remain in foil pouch with unused strips. Keep pouch sealed when not in use to maintain a dry environment. Remove excess air before sealing.
- 12 months after production (use within 2 months after opening the reaction plate and reagent bottles/vials).

5. Equipment and Materials Required but not Provided

- 100µl micropipette and tips: For dispensing reagents and samples
- 12-channel micropipette and tips (50~200µl): For dispensing reagents
- 12-channel micropipette tray: For reagent dispensing
- Orbital shaker
- Microplate washer (optional)
- Microplate reader (measurement wavelength 450nm): For absorbance measurement
- Distilled water: For preparing wash buffer

5. Sample Collection and Storage

5.1 The test can be performed with serum as well as with urine as samples. The applicability for analyzing specimens other than serum or urine remains to be verified.

5.2 Urine Samples: If turbidity or precipitates are observed, centrifuge at 2000~5000g for 10 minutes to obtain the supernatant as sample.

5.3 Serum Samples: Separate the serum as soon as possible after blood collection. To remove the influence of high-molecular-weight components in the serum, perform filtration using an ultrafiltration membrane with a molecular weight cutoff of 10,000.

5.4 Hemolytic, lipemic and icteric specimens should not be used.

6. Experimental Procedure

6.1 Separate the strips to be used by cutting through the protective tape with a sharp knife and transfer them to a strip frame. Pull off the protective tape from the strips intended for use. (Return the remaining strips to the plastic tray pack and reseal.)

6.2 According to the number of samples to be tested, add the Primary Antibody to the required volume of Assay Buffer at a ratio of 1:25, and mix thoroughly. This should be done within 60 minutes before use.

6.3 Pipette 50µL of the 8-OHdG calibrator (0.125, 0.25, 0.5, 1, 4, 10ng/mL) and samples into the bottom of each well.

6.4 Add 50µL of Primary Antibody solution to each well, incubate the frame for 90 minutes at room temperature on an orbital shaker at medium speed.

6.5 Discard or aspirate the contents of the wells and wash thoroughly with 300µl wash buffer diluted 10 times with distilled water, remove residual liquid by tapping the inverted plate on a clean absorbent paper. Repeat this 3 times. Alternatively, a washing device may be used.

6.6 Within 60 minutes before use, prepare the needed volume of Secondary Antibody solution by mixing 1.5mL Assay Buffer with 60µL Secondary Antibody stock solution (25×) per strip.

6.7 Dispense 100µL of Secondary Antibody solution into each well and incubate the frame for 60 minutes at room temperature on an orbital shaker at medium speed.

6.8 After incubation, wash the wells as in step 5.5. Ensure no residual wash buffer remains.

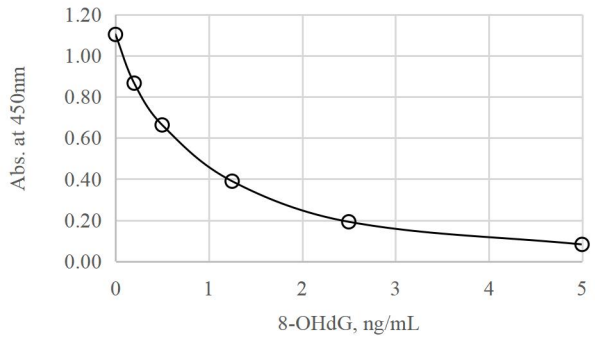
6.9 Dispense 100µL of Chromogenic Substrate solution into each well, and mix thoroughly. Incubate for 20 minutes at room temperature with slow shaking.

6.10 Add 100µL of Stop Solution into each well. Shake for at least 10 seconds on an orbital shaker.

6.11 Quantitation. Read the optical density at 450nm in a microplate photometer within 10 minutes. Plot absorbance vs. calibrator concentration to construct the 8-OHdG calibrator curve. When using analyzing software, it is recommended to use the Four Parameter Logistic (4PL) Regression (alternatively: cubic-spline or logit-log). The concentration of the controls and samples in ng/ml can be read directly from this calibration curve by using their average optical density.

7. Assay Characteristics

7.1 The typical calibration curve for the 8-OHdG ELISA is shown below.



7.2 Analytical sensitivity. The analytical sensitivity of the 8-OHdG ELISA is typically better than 0.079ng/mL (79pg/mL), if defined as the mean of the measurement values for the zero calibrator minus 2 standard deviations (mean value - 2SD).

7.3 Recovery. Spiked serum samples were prepared by adding varying levels of 8-OHdG to pooled serum specimens containing a known amount of 8-OHdG. Recoveries were in the range of 92 - 107% with a mean value of 102.2% (n=15).

7.4 Cross reactivity (at the 50% displacement level):

Substance	Cross Reactivity (%)
8-OHdG (8-oxodG)	100
8-OHG	0.12%
Guanosine	<0.01%
Uracil	<0.01%
Thymine	<0.01%
Guanine	<0.01%
Creatinine	<0.01%

7.5 Intra-assay Precision: ≤ 10%, Inter-assay Precision: ≤ 15%.

7.6 The range of measurement: 0.2 - 5.0ng/mL.

8. References

1) Ana-Maria Chiorcea-Paquim. 8-oxoguanine and 8-oxodeoxyguanosine Biomarkers of Oxidative DNA Damage: A Review on HPLC–ECD Determination. Molecules 2022, 27(5), 1620.
2) Athanasios Valavanidis, Thomais Vlachogianni and Constantinos Fiotakis. 8-hydroxy-2′ -deoxyguanosine (8-OHdG): A Critical Biomarker of Oxidative Stress and Carcinogenesis. Journal of Environmental Science and Health, Part C: Environmental Carcinogenesis and Ecotoxicology Reviews, 2009, 27, 120-139.
3) Kemal Sami Korkmaz, Bilge Debelec Butuner, Dirk Roggenbuck. Detection of 8-OHdG as a diagnostic biomarker. J Lab Precis Med 2018, 3, 95.

Developed and Manufactured by:

Shanghai Baiwei Biotechnology Co., Ltd.
Address: Unit C401-3, Building 7, Pujiang Wisdom Valley, No.1188 Lianhang Road, Pujiang Town, Minhang District, Shanghai, China.
URL: bowei-biotech.com
Contact: +8615221067868