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8-hydroxy-2'-deoxyguanosine (8-OHdG) ELISA Kit

rev 10/16

(Catalog # K4160-100, 100 assays, Store at 4°C)

I. Introduction:

8-hydroxy-2'-deoxyguanosine (8-OHdG) is an oxidized derivative of deoxyguanosine. Concentrations of 8-oxo-dG within a cell are a measurement of oxidative stress. It is considered as a critical biomarker of oxidative stress and carcinogenesis. This ELISA kit uses Competitive-ELISA as the method. The microtiter plate provided in this kit has been pre-coated with 8-OHdG. During the reaction,8-OHdG in the sample or standard competes with a fixed amount of 8-OHdG on the solid phase supporter for sites on the Biotinylated Detection Antibody specific to 8-OHdG. The concentration of 8-OHdG in the samples is determined by comparing the O.D. of the samples to the standard curve.

II. Application:

This ELISA kit I used for *in vitro* quantitative determination of 8-OHdG.
Detection Range: 1.563 - 100 ng/ml
Sensitivity: < 0.94 ng/ml

III. Specificity:

Universal

IV. Sample Type:

Serum, plasma, tissue homogenates and other biological fluids

V. Kit Contents:

Components	K4160-100	Part No.
Micro ELISA Plate	8 X 12 strips	K4160-100-1
Lyophilized Standard	2 vials	K4160-100-2
Sample / Standard dilution buffer	20 ml	K4160-100-3
Biotin- detection antibody (Concentrated)	60 µl	K4160-100-4
Antibody dilution buffer	10 ml	K4160-100-5
HRP-Streptavidin Conjugate (SABC)	120 µl	K4160-100-6
SABC dilution buffer	10 ml	K4160-100-7
TMB substrate (avoid light)	10 ml	K4160-100-8
Stop Solution	10 ml	K4160-100-9
Wash buffer (25X)	30 ml	K4160-100-10
Plate sealers	5	K4160-100-11

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Precision pipettes with disposable tips.
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

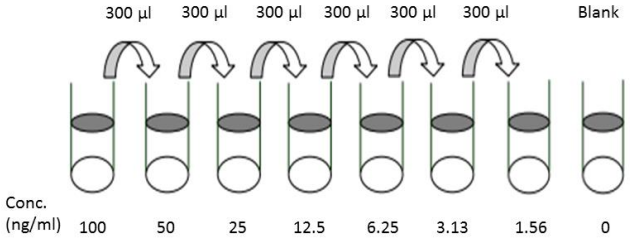
VII. Storage and Handling:

The entire kit may be stored at 4°C for up to 6 month from the date of shipment. Avoid freeze-thaw cycles.

VIII. Reagent Preparation:

Note: Prepare reagents within 30 minutes before the experiment.

1. **Biotin- detection antibody working solution**: Calculate the total volume of the working solution: 0.05 ml/well × quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the Biotin- detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly.
2. **HRP-Streptavidin Conjugate (SABC)**: Calculate the total volume of the working solution: 0.1 ml/well × quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly.
3. **Wash Buffer**: Dilute 30mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4°C.
4. **Standard Preparation:**
   - Reconstitute the lyophilized Human 8-OHdG standard by adding 1 ml of Standard/Sample Dilution Buffer to make the 100 ng/ml standard stock solution.
   - Allow solution to sit at room temperature for 10 minutes, then gently vortex to mix completely. Use within 2 hours of



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reconstituting.

- Prepare 0.6 ml of 50 ng/ml top standard by adding 0.3 ml of the above stock solution in 0.3 ml of Standard/Sample Dilution Buffer. Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay.
- Suggested standard points are: 50, 25, 12.5, 6.25, 3.13, 1.56 ng/ml

5. Sample Preparation:

Note: Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Homogenized samples are not suitable for use in this assay.

- **Serum**: Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000×g. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.
- **Plasma**: Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15 min at 4°C at 1000xg within 30 min of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.
- **Tissue homogenates**: Rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess hemolysis blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS with a glass homogenizer on ice. (The volume depends on the weight of the tissue. 9 mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) To further break the cells, sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000×g to retrieve the supernatant.
- **Cell culture supernatant**: Centrifuge supernatant for 20 minutes to remove insoluble impurity and cell debris at 1000×g at 4°C. Collect the clear supernatant and carry out the assay immediately or aliquot and store at -20°C.
- **Other biological fluids:** Centrifuge samples for 20 min at 1000×g at 4°C. Collect the supernatant and carry out the assay immediately.
- End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration falls the optimal detection range of the kit.

IX. Assay Protocol:

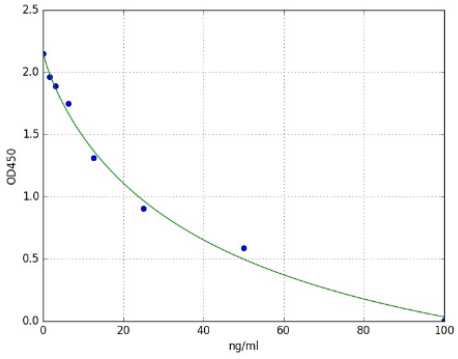
Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VIII.
2. Wash plate 2 times with **1X Wash Solution** before adding standard, sample and control wells.
3. Add 50 µl of each **standard** and **samples** into appropriate wells.
4. Immediately add 50 µL of **Biotinylated Detection Antibody working solution** to each well. Cover well and incubate for 45 minutes at 37ºC.
5. Discard the solution and wash 3 times with **1X Wash Solution**. Wash by filling each well with Wash Buffer (350 µl) using a multi-channel pipette or autowasher. Let it soak for 1-2 minutes, then remove all residual wash-liquid from the wells by aspiration. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent material.
6. Add 0.1 ml of **SABC working solution** into each well, cover the plate and incubate at 37ºC for 30 min.
7. Discard the solution and wash 5 times with **1X Wash Solution** as step 5.
8. Add 90 µl of **TMB substrate** into each well, cover the plate and incubate at 37 ºC in dark within 15-30 min. The shades of blue should be seen in the first 3-4 wells by the end of incubation.
9. Add 50 µl of **Stop Solution** to each well. Read result at 450 nm within 20 minutes.

X. CALCULATION:

Average the duplicate readings for each standard and samples. Create a standard curve by plotting the mean OD Value for each standard on the y-axis or x-axis against the concentration on the x-axis or y-axis and draw a best fit curve through the points on the graph. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it after appropriate dilution If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.



XI. RELATED PRODUCTS:

- 8-Hydroxy-2'-deoxyguanosine (Cat. No. 2177-1, -5)
- 3-Nitrotyrosine ELISA Kit (K4158-100)
- 8-Hydroxyguanosine (Cat. No. 2176-1, -5)
- 4-Hydroxynonenal (Cat. No. 2083-1,5)
- 3-Nitrotyrosine Antibody(39B6) (Cat. No. 5412-100)

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