



User's Manual

8-OHdG(8-Hydroxydeoxyguanosine) ELISA Kit

REF

DEIA-CL031



96T

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

8-OHdG ELISA Kit allows for the in vitro quantitative determination of 8-OHdG concentrations in serum, plasma, tissue homogenates and other biological fluids.

Principles of Testing

This kit was based on Competitive-ELISA detection method. The microtiter plate provided in this kit has been pre-coated with target. During the reaction, target in the sample or standard competes with a fixed amount of target on the solid phase supporter for sites on the Biotinylated Detection Antibody specific to target. Excess conjugate and unbound sample or standard are washed from the plate, and HRP-Streptavidin (SABC) is added to each microplate well and incubated. Then TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm. The concentration of target in the samples is then determined by comparing the OD of the samples to the standard curve.

Reagents And Materials Provided

No.	Item	Specifications(96T)	Storage
E001	ELISA Microplate(Dismountable)	8×12	2-8°C/-20°C
E002	Lyophilized Standard	2vial	2-8°C/-20°C
E039	Sample Dilution Buffer	20ml	2-8°C
E003	Biotin-labeled Antibody(Lyophilized)	1vial	2-8°C(Avoid Direct Light)
E005	Purified water	200ul	2-8°C
E040	Antibody Dilution Buffer	10ml	2-8°C
E034	HRP-Streptavidin Conjugate(SABC)	120ul	2-8°C(Avoid Direct Light)
E049	SABC Dilution Buffer	10ml	2-8°C
E024	TMB Substrate	10ml	2-8°C(Avoid Direct Light)
E026	Stop Solution	10ml	2-8°C
E038	Wash Buffer(25X)	30ml	2-8°C
E006	Plate Sealer	3/5pieces	
E007	Product Description	1copy	

Stability

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 10% within the expiration date under appropriate storage condition.

Standard(n=5)	37°C for 1 month	2-8°C for 6 months
Average (%)	80	95-100

To minimize extra influence on performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is strongly suggested that the same operator performs the whole assay from the beginning to the end.

Materials Required But Not Supplied

1. Microplate reader (wavelength:450nm)
2. 37°C incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Storage

2-8°C for 6 months

Specimen Collection And Preparation

Serum: The concentration of free 8-OHdG in plasma is very low relative to the level of DNA-incorporated 8-OHdG. Glomerular filtration results in excretion of 8-OHdG into the urine, while the DNA-incorporated 8-OHdG remains in the blood. The differing fates of free versus DNA-incorporated 8-OHdG should be considered in experimental design. 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.

Cultured Suspension Cells: Grow $1-5 \times 10^6$ cells in suspension using complete medium in a suitable tissue culture plate or flask. Count the cells. Harvest cells by centrifugation and remove growth medium. Wash one time with 1X PBS. Suspend cell pellets at 1×10^6 cells/mL in ice-cold 1X PBS. For example, add 5 mL 1x PBS to 5×10^6 cells. Aliquot 1 mL into 1.5 mL microcentrifuge tubes. Centrifuge at $10,000 \times g$ for 10 seconds at 2-8 °C. Discard supernatant. Proceed to DNA Extraction. (Cell pellets can be flash frozen in liquid nitrogen and stored at $\leq -70^\circ\text{C}$ for later use.)

Cultured Adherent Cells: Grow $1-5 \times 10^6$ adherent cells in complete medium in a suitable tissue culture dish or flask until 75% confluent. Remove the growth medium and harvest cells by trypsinization or a method of choice. Count the cells. Wash one time with 1X PBS. Suspend the cell pellets at 1×10^6 cells/mL in ice-cold 1X PBS. For example, add 5 mL 1X PBS to 5×10^6 cells. Aliquot 1 mL into 1.5 mL microcentrifuge tubes.

Centrifuge at 10,000 x g for 10 seconds at 2-8°C. Discard supernatant. Proceed to DNA Extraction. (Cell pellets can also be flash frozen in liquid nitrogen and stored at $\leq -70^{\circ}\text{C}$ for later use).

Tissue Samples: The tissue specimen should be cut into a 2 mm cube and weigh approximately 8-12 mg in mass. Proceed to DNA Extraction.

DNA Extraction: Extract DNA from the above cultured cells or tissue samples by a desired method or commercial extraction kit. (Generally the minimal amount of extracted DNA required for each sample is 20-50 μg .) Quantitate DNA spectrophotometrically ($\text{OD}_{260} = 50\mu\text{g/mL}$). The suggested final DNA concentration is 200 $\mu\text{g/mL}$ to 1000 $\mu\text{g/mL}$. Add 100X Cations to DNA solution for final 1X concentration. Add 2ul DNase I per 50 μg DNA and incubate for 1 hour at 37°C. Add 2ul Alkaline Phosphatase per 50 μg DNA and incubate 1 hour at 37°C. Assay immediately or aliquot and store at $\leq -20^{\circ}\text{C}$.

Plasma Samples: Withdraw blood according to standard procedures using Sodium Heparin or EDTA as anticoagulant. Collect plasma by centrifugation at room temperature in a horizontal rotor (swinging bucket) in a proper adaptor for 1 minutes at 1,500 x g within 30 minutes of blood collection. Carefully transfer the plasma to a new centrifuge tube and centrifuge for another 15 minutes at 2500 x g at room temperature. Carefully transfer the supernatant and assay immediately or store at $\leq -20^{\circ}\text{C}$ in aliquots for later use. Avoid repeated freeze-thaw cycles

Urine Samples: Collect urine according to standard procedure into a sterile container. To clarify, centrifuge 2,000 x g for 15 minutes, or filter using a 0.45 μm filter to remove precipitate. Assay immediately or store at $\leq -20^{\circ}\text{C}$ in aliquots for later use. Avoid repeated freeze-thaw cycles.

Saliva Samples: Collect saliva according to standard procedure in a centrifuge tube. To clarify, centrifuge at 2,000 x g for 15 minutes. Carefully remove supernatant and assay immediately or store at $\leq -20^{\circ}\text{C}$ in aliquots for later use. Avoid repeated freeze-thaw cycles.

Note: Samples to be used within 5 days can be stored at 2-8°C, besides that, samples must be stored at -20°C (assay ≤ 1 month) or -80°C (assay ≤ 2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles. The hemolytic samples are not suitable for this assay.

Sample Dilution

The user should estimate the concentration of target protein in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be making in pre-experiment. If samples with very high concentrations, dilute samples with PBS first and then dilute the samples with Sample Dilution.

Reagent Preparation

Bring all reagents and samples to room temperature for 20 minutes before use.

Wash Buffer

If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely been dissolved. The solution should be cooled to room temperature before use.

Dilute 30ml Concentrated Wash Buffer into 750ml Wash Buffer with deionized or distilled water. Put unused solution back at 2-8°C.

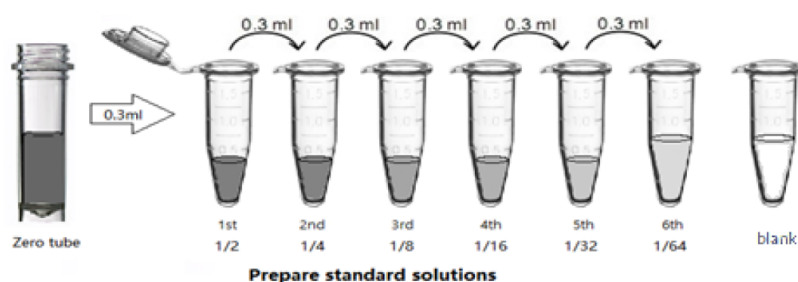
Standards

- 1) Add 1 ml Sample Dilution Buffer into one Standard tube (labeled as zero tube), keep the tube at room temperature for 10 minutes and mix them thoroughly.

Note: If the standard tube concentration higher than the range of the kit, please dilute it and labeled as zero tube.

- 2) Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3ml of the Sample Dilution Buffer into each tube. Add 0.3ml of the above Standard solution (from zero tube) into 1st tube and mix them thoroughly. Transfer 0.3ml from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3ml from 2nd tube to 3rd tube and mix them thoroughly, and so on. Sample Dilution Buffer was used for the blank control.

Note: It is best to use Standard Solutions within 2 hours.



Preparation of Biotin-labeled Antibody Working Solution

Prepare it within 1 hour before experiment.

- 1) Dissolve: Add 70ul purified water into tube and mix them thoroughly, after the biotin-labeled antibody is dissolved, please store it at 2-8°C.
- 2) Calculate required total volume of the working solution: 50ul /well × quantity of wells. (Allow 0.1-0.2ml more than the total volume.)
- 3) Dilute the Biotin-detection antibody with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1ul Biotin-labeled antibody into 99ul Antibody Dilution Buffer.)

Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution

Prepare it within 30 minutes before experiment.

- 1) Calculate required total volume of the working solution: 0.1ml/well × quantity of wells. (Allow 0.1-0.2ml more than the total volume.)
- 2) Dilute the SABC with SABC Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1ul of SABC into 99ul of SABC Dilution Buffer.)

Assay Procedure

Washing Notes

Manual: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material.

Automatic: Aspirate all wells, and then wash plate with 350ul wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer shall be set for soaking 1 minute. (**Note:** set the height of the needles; be sure the fluid can be sipped up completely)

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37°C. It is recommended to plot a standard curve for each test.

1. **Set standard,** test samples, control (blank) wells on the pre-coated plate respectively, and then, records their positions. It is recommended to measure each standard and sample in duplicate. Wash plate 2 times before adding standard, sample and control (blank) wells!
2. **Add Sample and Biotin-labeled Antibody:** Add 50ul of Standard, Blank, or Sample per well. The blank well is added with Sample/Standard Dilution Buffer. Immediately add 50ul Biotin-labeled Antibody Working Solution into each well. Cover with the Plate sealer we provided. Gently tap the plate to ensure thorough mixing. Incubate for 45 minutes at 37°C. (Solutions are added to the bottom of microplate well, avoiding inside wall touching and foaming as much as you can.)
3. **Wash:** Remove the cover, and wash plate 3 times with Wash Buffer, and let the wash buffer stay in the wells for 1 minute each time. After the last wash, remove any remaining Wash Buffer by aspirating or decanting.
4. **HRP-Streptavidin Conjugate (SABC):** Add 100ul SABC Working Solution into each well. Cover it with a new Plate sealer. Incubate for 30 minutes at 37°C.
5. **Wash:** Remove the cover and wash plate 5 times with Wash Buffer, and let the wash buffer stay in the wells for 1-2 minutes each time.
6. **TMB Substrate:** Add 90ul TMB Substrate into each well, cover the plate and incubate at 37°C in dark within 10-20 minutes. (Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells.)
7. **Stop:** Add 50ul Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.
8. **OD Measurement:** Read the O.D. absorbance at 450nm in Microplate Reader immediately after adding the stop solution. Regarding calculation, the standard curve can be plotted as the O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Summary

Step1: Wash plate 2 times before adding Standard, Sample and Control (blank) wells!

Step2: Add 50ul Standard or Sample into each well. Immediately add 50ul Biotin-labeled Antibody into each well, gently tap the plate to ensure thorough mixing then incubate for 45 minutes at 37°C.

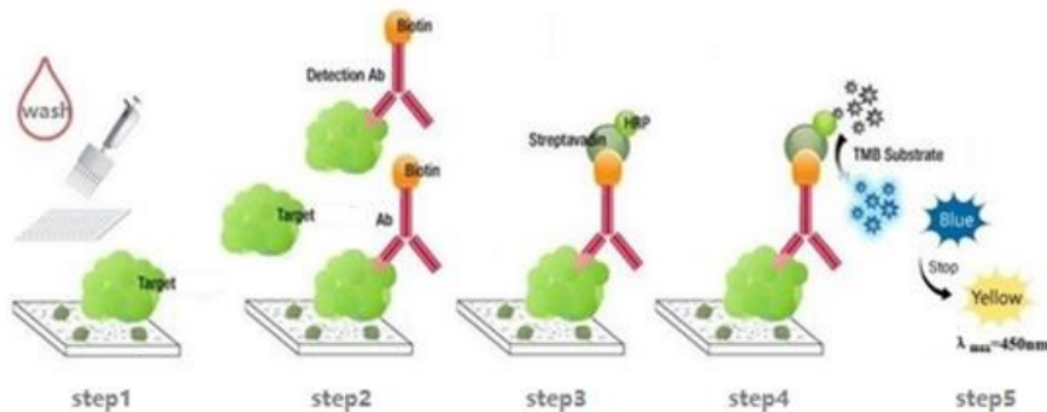
Wash step: Aspirate and wash plates 3 times.

Step3: Add 100ul SABC Working Solution into each well and incubate for 30 minutes at 37°C.

Wash step: Aspirate and wash plates 5 times.

Step4: Add 90ul TMB Substrate Solution. Incubate 10-20 minutes at 37°C.

Step5: Add 50ul Stop Solution. Read at 450nm immediately and calculation.

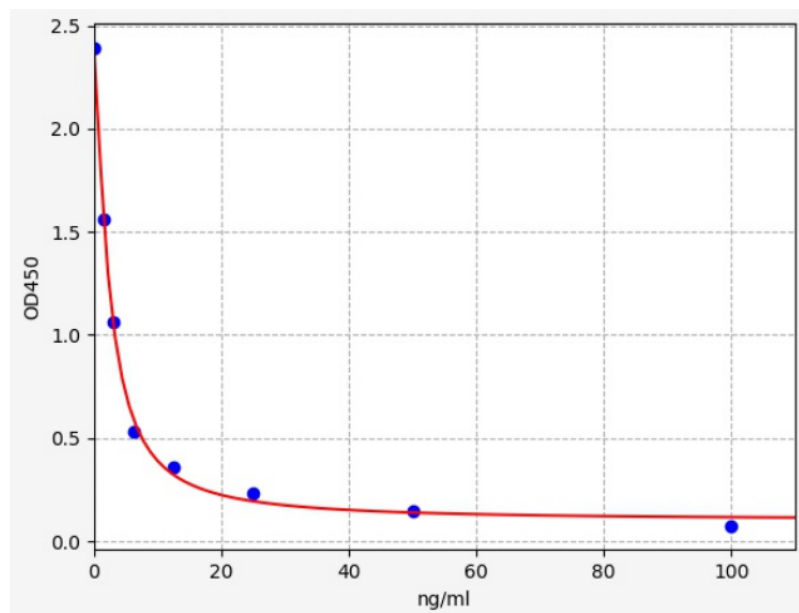


Calculation

Regarding calculation, the standard curve can be plotted as the O.D.450 of each standard solution (Y) vs, the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve. It is recommended to use some professional software to do this calculation, such as Curve Expert 1.3 or 1.4.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Typical Standard Curve



Precision

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Detection Range

1.563-100ng/ml

Sensitivity

< 0.938ng/ml

Specificity

This assay has high sensitivity and excellent specificity for detection of 8-OHdG. No significant cross-reactivity or interference between 8-OHdG and analogues was observed.

Note: Limited by current skills and knowledge, it is difficult for us to complete the cross-reactivity detection between 8-OHdG and all the analogues, therefore, cross reaction may still exist.

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of 8-OHdG and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8
serum(n=5)	90-102%	88-105%	86-105%
EDTA plasma(n=5)	82-101%	88-101%	89-96%
heparin plasma(n=5)	83-99%	83-94%	81-99%

Recovery

Matrices listed below were spiked with certain level of 8-OHdG and the recovery rates were calculated by comparing the measured value to the expected amount of 8-OHdG in samples.

Matrix	Recovery range(%)	Average(%)
serum(n=5)	89-105	96
EDTA plasma(n=5)	88-96	93
heparin plasma(n=5)	88-105	95

Precautions

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive and high background.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don't let microplate dry at the assay, for dry plate will inactivate active components on plate.
8. Don't reuse tips and tubes to avoid cross contamination.
9. Avoid using the reagents from different batches together.