ab201734 8-hydroxy 2 deoxyguanosine ELISA Kit

For the competitive quantitative measurement of 8-hydroxy 2 deoxyguanosine (8 OH-dG) in urine, cell culture, plasma and other sample matrices. This product is for research use only and is not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab201734
(use abcam.cn/ab201734 for China, or abcam.co.jp/ab201734 for Japan)

Materials Supplied and Storage

Kit has a storage time of 1 year from receipt, this kit will perform as specified if the components are stored as directed. All reagents are stable as supplied at 4°C, except the standard, detection antibody and the plate, which should be stored at -20°C. For optimum storage, the 8-OHdG Standard should be aliquoted into smaller portions and then stored appropriately. Avoid repeated freeze/thaw cycles (10 µL of Standard can prepare a triplicate standard curve).

Item	Quantity	Storage Temp.
8-hydroxy-2-deoxyguanosine: BSA Coated Plate	96 wells	-20°C
8-hydroxy-2-deoxyguanosine Standard (Stock)	1 vial (100 µL)	-20°C
8-hydroxy-2-deoxyguanosine HRP Conjugated Monoclonal Antibody	1 vial (75 µL)	-20°C
Sample and Standard Diluent (Red)	1 vial (50 mL)	4°C
8-hydroxy-2-deoxyguanosine Antibody Diluent (Blue)	1 vial (13 mL)	4°C
Wash Buffer Concentrate (10X)	1 vial (50 mL)	4°C
TMB Substrate	1 vial (13 mL)	4°C
Stop Solution	1 vial (13 mL)	4°C
Plate Cover	2	4°C

Materials Required, Not Supplied:

- A plate reader capable of measuring absorbance at 450 nm.
- Deionized or distilled water
- 2. Adjustable pipettes and a repeat pipettor.
- Materials used for Sample Preparation.

1. Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. Prepare only as much reagent as is needed on the day of the experiment.

1. **1X Wash Buffer:**
   Prepare 1X Wash buffer by diluting 10X Wash Buffer in distilled or deionized water. Store reconstituted 1X Wash Buffer at +2-8°C for up to one (1) month. Do not use 1X Wash Buffer if it becomes visibly contaminated during storage.
2. **1X 8-hydroxy-2-deoxyguanosine: HRP Conjugate Monoclonal Antibody:**
   Determine the amount of Antibody Preparation required. For every strip-well used (8-wells), prepare 0.5 mL of Antibody Preparation.
   Prepare Antibody Preparation by diluting the 8-hydroxy-2-deoxyguanosine: HRP Conjugate Antibody Concentrate 1:100 with 8-hydroxy-2-deoxyguanosine Antibody Diluent. For example, if 6 mL of Antibody Preparation is required (one whole plate), dilute 60 µL of Antibody in 6 mL of 8-hydroxy-2-deoxyguanosine Antibody Diluent. Mix well prior to use.
3. **TMB and Stop Solution:** Provided at working strength

2. Standard Preparation

Always prepare a fresh set of standards for every use. Discard after use.
Δ Note The standard should be aliquoted into portions before use to ensure product integrity. Avoid freeze/thaw cycles. (10 µL of standard can prepare a triplicate standard curve).

2.1 Centrifuge the 8-hydroxy-2-deoxyguanosine Standard (Stock) vial before removing the cap. This process with assure that all of the standard is collected and available.
2.2 Label eight tubes with Standards #1 – 8.
2.3 Add 500 µL of Sample and Standard Diluent to Tube #1.
2.4 Add 250 µL of Sample and Standard Diluent to Tube #2 - #7.
2.5 Add 500 µL of Sample and Standard Diluent to Tube #8.
2.6 Add 10 µL of the 3.06 µg/mL 8-hydroxy-2-deoxyguanosine Standard to Tube #1 for a concentration of 60 ng/mL. Mix well.
2.7 Transfer 250 µL from Tube #1 to Tube #2. Mix well.
2.8 Using the table below as a guide, repeat for Tubes #4 - #7.

Standard #	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	Stock	10	500	3060	60
2	Standard #1	250	250	60	30
3	Standard #2	250	250	30	15
4	Standard #3	250	250	15	7.5
5	Standard #4	250	250	7.5	3.75
6	Standard #5	250	250	3.75	1.875
7	Standard #6	250	250	1.875	0.94
8	Diluent	0	500	0	0

3. Sample Preparation

General Precautions: All samples must be free of organic solvents prior to assay. Samples that cannot be assayed immediately should be stored as indicated below. Please be advised that all suggested dilutions below are simply recommended as a starting point, and it may be necessary to adjust the dilution based on experimental results.

3.1 Plasma/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.
Storage: Collect plasma using established methods and store at -80°C.
Dilution: Serum samples may be diluted 1:20 (v:v) in Sample and Standard Diluent as the starting dilution prior to testing.

3.2 Cell Lysates

Storage: Collect lysates using established methods and store at -80°C until use.
Usage: Purify DNA using a commercially available extraction kit. Digest DNA using nuclease P1 following the manufacturer's instructions. Adjust pH to 7.5 - 8.5 using 1M Tris. Add 1 unit of alkaline phosphatase per 100 µg of DNA and incubate at 37°C for 30 minutes. Boil for 10 minutes and place on ice until use.

3.3 Urine

Interference in urine is infrequent; dilutions appropriate for this assay show a direct linear correlation between 8-OHdG immunoreactivity and 8-OHdG concentration. Urinary

concentrations of 8-OH-dG can vary considerably and can be standardized against creatinine levels if required.
Storage: Fresh urine samples should be centrifuged at 2,000 x g for 10 minutes or filtered with a 0.2µm filter before this assay and stored at -20°C immediately after collection.
Dilution: Dilute urine samples 1:20 (v:v) in Sample and Standard Diluent as the starting dilution prior to testing. For example: 9 µL of sample into 171 µL of Sample and Standard Diluent.

3.4 Culture Media Samples

Storage: Collect culture media samples and store at -80°C.
Dilution: Foetal bovine serum contains 8-OHdG, therefore assays should either be performed in serum-free medium or PBS if these samples are assayed directly. If the 8-OHdG concentration is high enough to dilute the sample 10-fold with Sample and Standard Diluent, the assay can be performed without any modifications. When assaying less concentrated samples (where samples cannot be diluted 1:10 with Sample and Standard Diluent), dilute the standards in the same culture medium as that used for the experiment. This will ensure that the matrix for the standards is comparable to the samples. We recommend that a standard curve be run first to ensure that the assay will perform in a particular culture medium.

3.5 Tissue Samples

Storage: Snap-freeze tissue samples in liquid nitrogen immediately after collection. Store at -80°C until use.
Usage: When ready to use the samples, thaw and add 5 mL of homogenization buffer (0.1 M phosphate buffer, pH 7.4, containing 1 mM EDTA) per gram of tissue. Homogenize the sample using either a Polytron-type homogenizer or a sonicator. Centrifuge at 1,000 x g for 10 minutes and purify the supernatant using a commercially available DNA extraction kit. Digest DNA using nuclease P1 following the manufacturer's instructions. Adjust the pH to 7.5-8.5 using 1 M Tris. Add 1 unit of alkaline phosphatase per 100 µg of DNA and incubate at 37°C for 30 minutes. Boil for 10 minutes and place on ice until use.

3.6 Saliva

Storage: Saliva samples should be stored at -80°C immediately after collection. Samples may be assayed directly after appropriate dilution.
Dilution: Saliva samples can be prepared 1:8 (v:v) in Sample and Standard Diluent as a suggested starting dilution.

4. Assay Procedure:

Use materials and reagents at room temperature. Assay all in duplicate.

4.1 Add 50 µL (in triplicate) of each of the following to appropriate wells:
- Prepared 8-hydroxy-2-deoxyguanosine Standard (Tube #1 - Tube #7) into wells labelled S1-S7
- Zero Standard (Tube #8- Sample and Standard Diluent, which represents 0 ng/mL) into wells labelled S8
- Samples (previously prepared- See Sample Preparation) into wells labelled 1-23

4.2 Add 50 µL of the previously diluted 8-hydroxy-2-deoxyguanosine Antibody Preparation to each well, except the blank.
4.3 Add 50 µL of Standard and Sample Diluent and 50 µL of Antibody Diluent into wells labelled as the blank.
4.4 Cover each plate with the plate cover and incubate 1 hour at room temperature (+20-25˚C).
4.5 Carefully remove adhesive plate cover. Gently squeeze the long sides of the plate frame before washing to ensure all strips remain securely in the frame.
4.6 Empty plate contents. Use a multi-channel pipette or a plate washer to fill each well completely (300 µL) with 1X Wash buffer, then aspirate plate contents. Repeat procedure three additional times, for a total of FOUR washes. Blot plate by tapping gently onto paper towels or other absorbent material.

Δ Note Follow the same procedure when using an automated plate washer as well. Take care to avoid microbial contamination of equipment. Automated plated washers can easily become contaminated thereby causing assay variability.

Pipetting Summary Table

Well	Standard OR Sample Preparation	Standard & Sample Diluent	Antibody Preparation	Antibody Diluent	Total Volume/ Well
Standard(S1-S7)	50 µL	Inc. in Standard Preparation	50 µL	Inc. in Antibody Preparation	100 µL
Zero Standard(S8)		50 µL	50 µL	Inc. in Antibody Preparation	100 µL
Blank		50 µL		50 µL	100 µL
Samples(1-23)	50 µL	Inc. in sample Preparation	50 µL	Inc. in Antibody Preparation	100 µL

Δ Note Only remove the required amount of TMB Substrate and Stop Solution for the number of strips being used. Do NOT use a glass pipette to measure the TMB Substrate solution. Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.

4.7 Add 100 µL of TMB Substrate into each well.
4.8 Cover carefully with the second provided plate cover.
4.9 Allow the enzymatic color reaction to develop at room temperature (+20-25°C) in the dark for 30 minutes. The substrate reaction yields a blue solution.
4.10 After 30 minutes, carefully remove the plate cover, and stop the reaction by adding 100 µL of Stop Solution to each well. Tap plate gently to mix. The solution in the wells should change from blue to yellow.
Δ Note Evaluate the plate within 30 minutes of stopping the reaction.
4.11 Wipe underside of wells with a lint-free tissue.
4.12 Measure the absorbance on the ELISA plate reader set at 450 nm.

5. Calculations

Many plate readers come with data reduction software that plot data automatically.

Preparation of the Data

Δ Note If the plate reader has not subtracted the absorbance readings of the blank wells from the absorbance readings of the rest of the plate, be sure to do that now.

5.1 For each sample and standard, subtract the average blank absorbance value (OD) and calculate the average for each of the replicates.
5.2 Plot the average absorbance versus 8-OHdG concentration of the standards. Sample concentrations may be extrapolated from the standard curve using the blank subtracted average absorbance values.
5.3 Samples with absorbance values outside of the standard curve should be measured again using a dilution which will bring the absorbance into the range of the standard curve. Remember to use the dilution factor which calculating the concentration present in the original sample.

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