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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.

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1. Overview

Abcam's 8-hydroxy 2 deoxyguanosine (8 OH-dG) *in vitro* ELISA Kit (ab201734) is designed for the competitive quantitative measurement of 8 OH-dG in urine, cell culture, plasma and other sample matrices.

The ELISA utilizes an 8-hydroxy-2-deoxy Guanosine-coated plate and an HRP-conjugated antibody for detection which allows for an assay range of 0.94 - 60 ng/mL, with a sensitivity of 0.59 ng/mL. The other highlights of this kit are a quick incubation time of 60 minutes, stable reagents, and an easy to use protocol.

It is important to note that the 8-OHdG antibody used in this assay recognizes both free 8-OHdG and DNA-incorporated 8-OHdG. Since complex samples such as plasma, cell lysates, and tissues are comprised of mixtures of DNA fragments and free 8-OHdG, concentrations of 8-OHdG reported by ELISA methodology will not coincide with those reported by LC-MS where the single nucleoside is typically measured. This should be kept in mind when analyzing and interpreting experimental results.

8-hydroxy-2-deoxy Guanosine (8-OHdG) is produced by the oxidative damage of DNA by reactive oxygen and nitrogen species and is an established marker of oxidative stress. Hydroxylation of guanosine occurs in response to both normal metabolic processes and a variety of environmental factors (i.e., any biological process which increases reactive oxygen and nitrogen species). Increased levels of 8-OHdG are associated with aging as well as with a number of pathological conditions including cancer, diabetes, and hypertension.

In complex samples such as plasma, cell lysates, and tissues, 8-OHdG can exist as either the free nucleoside or incorporated in DNA. Once the blood enters the kidney, free 8-OHdG is readily filtered into the urine, while larger DNA fragments remain in the bloodstream. Because of the complexity of plasma samples, urine is a more suitable matrix for the measurement of free 8-OHdG than plasma. Urinary levels of 8-OHdG range between 2.7- 13 ng/mg creatine, while plasma levels of free 8-OHdG have been reported to be between 4-21 pg/mL as determined by LC-MS.

2. Protocol Summary

Remove appropriate number of 8-OHdG pre-coated well strips. Equilibrate all reagents to room temperature. Prepare all the reagents, standards and samples as instructed.

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Add HRP conjugated 8-OHdG antibody and either standard or sample to appropriate wells.

⇩

Wash to remove all unbound reagents.

⇩

Develop the well with TMB.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

For optimum storage, the 8-OHdG Standard should be aliquoted into smaller portions and then stored at -20°C. Avoid repeated freeze/thaw cycles (10 µL of Standard can prepare a triplicate standard curve).

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
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)	-20°C
8-hydroxy-2-deoxyguanosine Antibody Diluent (Blue)	1 vial (13 mL)	-20°C
Wash Buffer Concentrate (10X)	1 vial (50 mL)	-20°C
TMB Substrate	1 vial (13 mL)	-20°C
Stop Solution	1 vial (13 mL)	-20°C
Plate Cover	2	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- A plate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes and a repeat pipettor.
- A source of 'UltraPure' water. Water used to prepare all reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for this assay.
- Materials used for Sample Preparation.

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Kit components should be stored as indicated. All the reagents should be equilibrated to room temperature before use.
- Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross-contamination.
- Thoroughly mix the reagents and samples before use by agitation or swirling.
- All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
- Use a new adhesive plate cover for each incubation step.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- The use of assay reagents not provided in this kit or amendments to the protocol can compromise the performance of this assay.
- The components in each kit lot number have been quality assured and warranted in this specific combination only; please do not mix them with components from other kit lot numbers.
- If samples generate greater values than the highest standard, the samples should be re-assayed at a higher sample dilution. Similarly, if samples generate lower values than the lowest standard, the samples should be re- assayed at a lower sample dilution.
- Exact conditions may vary from assay to assay, a standard curve should be generated for every assay performed.
- Bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.

- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh 1X Wash Buffer. Do not allow wells to sit uncovered or dry for extended periods.

9. 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.

  Δ Note Water used to prepare all reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for this assay.

9.1 1X Wash Buffer

Prepare 1X Wash buffer by diluting 10X Wash Buffer in distilled or deionized water.
For example, if preparing 500 mL of 1X Wash Buffer, dilute 50 mL of 10X Wash Buffer into 450 mL of distilled water. Mix well.
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.

9.2 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.

9.3 TMB and stop solution have been provided at working strength.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

  Δ Note The Standard should be aliquoted into smaller portions before use to ensure product integrity. Avoid freeze/thaw cycles. (10 µL of Standard can prepare a triplicate standard curve).

10.1 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.

10.2 Label eight tubes with Standards #1 – 8.

10.3 Add 500 µL of Sample and Standard Diluent to Tube #1.

10.4 Add 250 µL of Sample and Standard Diluent to Tube #2 - #7.

10.5 Add 500 µL of Sample and Standard Diluent to Tube #8.

10.6 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.

10.7 Transfer 250 µL from Tube #1 to Tube #2. Mix well.

10.8 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

11. Sample Preparation

- Urine, cell culture, plasma are suitable sample matrices for use in the assay.
- Proper sample storage and preparation are essential for consistent and accurate results. Please read this section thoroughly before beginning the assay.
- 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.

11.1 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.

11.2 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.

11.3 Urine

Interference in urine is infrequent; dilutions appropriate for this assay show a direct linear correlation between 8-OHdG immunoreactivity and 8-OHdG concentration (see figure 1). 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.

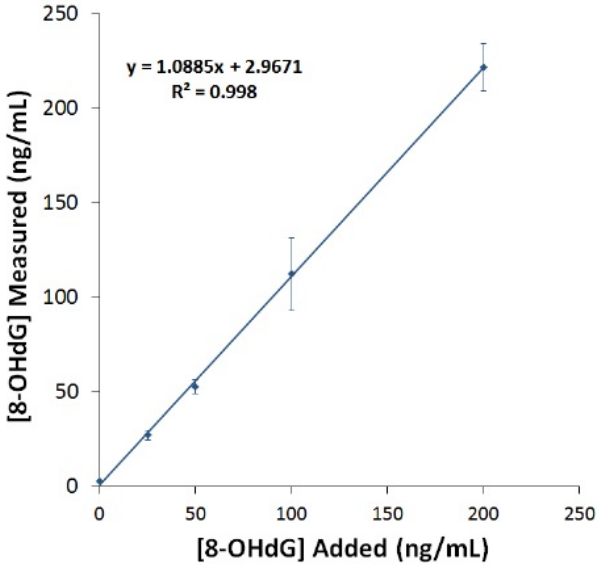

Figure 1. Recovery of 8-hydroxy-2-deoxy Guanosine from urine

Urine samples were spiked with 8-OHdG, diluted as described in the **Sample Preparation** section and analyzed using the 8-OHdG ELISA Kit. The y-intercept corresponds to the amount of 8-OHdG in un-spiked urine. Error bars represent standard deviations obtained from multiple dilutions of each sample.

11.4 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.

11.5 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.

11.6 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.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For statistical purposes, we recommend assaying samples in triplicate.
- A suggested plate format is shown in Figure 2, below. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- Pipetting Hints: Use different tips to pipette the buffer, standard, sample, tracer, and antibody. Before pipetting each reagent, equilibrate the pipette tip in that reagent (i.e., slowly fill the tip and gently expel the contents, repeat several times). Do not expose the pipette tip to the reagent(s) already in the well.
- Addition of the Reagents (please refer to Pipetting Summary Table below).

13.1 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

13.2 Add 50 µL of the previously diluted 8-hydroxy-2-deoxyguanosine Antibody Preparation to each well, except the blank.

13.3 Add 50 µL of Standard and Sample Diluent and 50 µL of Antibody Diluent into wells labelled as the blank.

13.4 Cover each plate with the plate cover and incubate 1 hour at room temperature (+20- 25˚C).

13.5 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.

13.6 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	Included in Standard Preparation	50 µL	Included in Antibody Preparation	100 µL
Zero Standard (S8)		50 µL	50 µL	Included in Antibody Preparation	100 µL
Blank		50 µL		50 µL	100 µL
Samples (1-23)	50 µL	Included in Sample Preparation	50 µL	Included 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.

13.7 Add 100 µL of TMB Substrate into each well.

13.8 Cover carefully with the second provided plate cover.

13.9 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.

13.10 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.

13.11 Wipe underside of wells with a lint-free tissue.

13.12 Measure the absorbance on the ELISA plate reader set at 450 nm.

14. Calculations

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

Preparation of the Data

The following procedure is recommended for preparation of the data prior to graphical analysis.

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.

14.1 For each sample and standard, subtract the average blank absorbance value (OD) and calculate the average for each of the replicates.

14.2 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.

14.3 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.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

Development conditions and the purity of the water used can substantially alter your results from the data presented below.

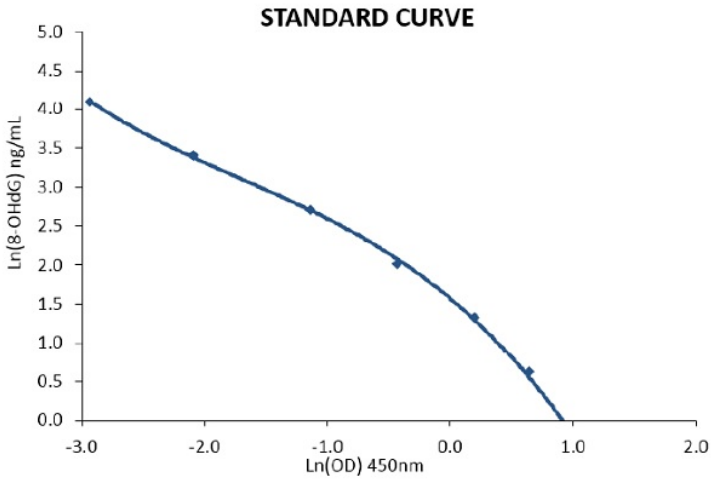

Figure 3. This standard curve was generated using the 8-OHdG ELISA Kit Protocol and is typical of the performance of this assay. This standard curve is for demonstration only. A fresh standard curve must be generated for each assay.

The intra- and inter-assay CV's have been determined and can be seen below.

Sample numbers	Assay replicates	n	Intra-assay variation (CV %)	Inter-assay variation (CV %)
3	30	90	<5	<5

Table 1. Intra- and inter-assay variation. %CV represents the variation in concentration (not absorbance) as determined using three samples of known concentration which were assayed thirty times on one plate (Intra-assay variation) and 30 times in 3 individual assays (Inter-assay variation).

16. Assay Specificity

Specificity of the 8-hydroxy-2-deoxyguanosine Monoclonal Antibody:

Compound	Cross-reactivity (%)
8-hydroxy-2-deoxyguanosine	100
8-hydroxy guanosine	23
8-hydroxy guanine	23
Guanosine	<0.01

SENSITIVITY-

The calculated minimal detectable (MDD) dose is 1 pg/mL. The MDD was determined by calculating the mean of zero standard replicates.

Please contact our Technical Support team for more information.

17. Troubleshooting

Problem	Possible Causes	Solutions
Poor Standard Curve	Improper standard solution	Confirm dilutions are made correctly.
	Standard degraded	Store and handle standard as recommended
	Curve doesn't fit scale	Try plotting using different scales
	Pipetting Error	Use calibrated pipettes and proper pipetting technique.
No Signal	Plate washings too vigorous	Check and ensure correct pressure in automatic wash system. Pipette wash buffer gently if washes are done manually.
	Wells dried out	Do not allow wells to dry out. Cover the plate for incubations.
	Target present below detection levels of kit	The basic range of DNA to use, if the damage is low, 100 ug/ml - if maximally damaged, 1 ng/ml and dilute from there.
High Background	Wells are insufficiently washed	Wash wells as per protocol
	Contaminated wash buffer	Prepare fresh wash buffer
	Waiting too long to read the plate after adding stop solution	Read plate immediately
Low sensitivity	Standard is degraded	Replace standard
	Mixing or substituting reagents from other kits	Avoid mixing components

18. Notes

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