

DNA Damage (8-OHdG) ELISA Kit



Catalog #9143

1 x 96-well plate/kit

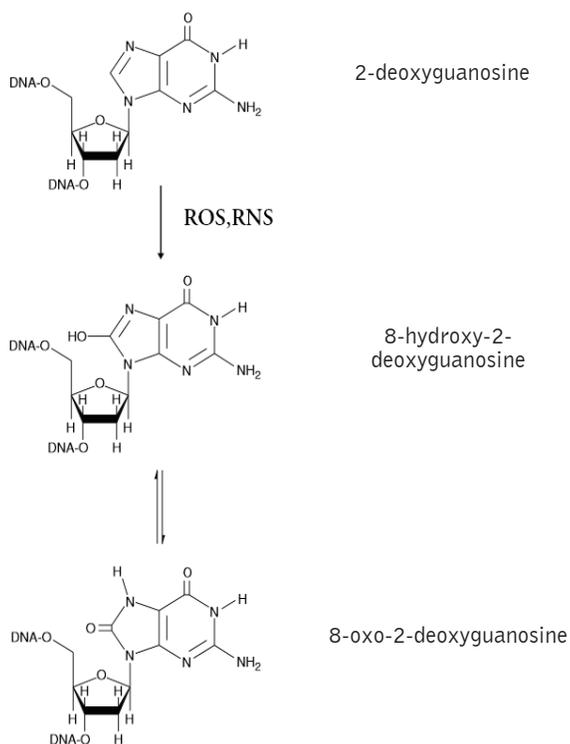
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1. INTRODUCTION

8-hydroxy-2-deoxyguanosine (8-OHdG) is produced by the oxidative damage of DNA (Figure 1) by reactive oxygen and nitrogen species and serves as an established marker of oxidative stress.¹⁻⁴ Hydroxylation of guanosine occurs in response to both normal metabolic processes and a variety of environmental factors (*i.e.*, anything that increases reactive oxygen and nitrogen species). Increased levels of 8-OHdG are associated with the aging process as well as with a number of pathological conditions including cancer, diabetes, and hypertension.⁵⁻⁹

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.¹⁰⁻¹¹

Figure 1: Oxidation of Guanosine



ICT's DNA Damage (8-OHdG) ELISA is a competitive assay that can be used for the quantification of 8-OHdG in urine, cell culture, plasma, and other sample matrices. The ELISA utilizes an 8-hydroxy-2-deoxyguanosine-coated plate and an HRP-conjugated 8-OHdG specific detection antibody. This produces an assay with a useful range of 0.94 - 60 ng/mL, and a sensitivity of 0.59 ng/mL. Other highlights of this kit include a quick incubation time (60 minutes), stable reagents, and a user-friendly 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.

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Not for use in diagnostic procedures.**

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2. KIT CONTENTS

- 8-hydroxy-2-deoxyguanosine: BSA Coated 96-Well Plate, part #270.
- 2 Plate Covers, part #271
- 1 vial of 8-hydroxy-2-deoxyguanosine Standard (100 μ L at 3.06 μ g/mL), part #6646.
- 1 vial of 8-OHdG HRP Conjugated Monoclonal Antibody (75 μ L), part #6647.
- 1 bottle of Sample and Standard Diluent (50 mL), part #6648.
- 1 bottle of 8-hydroxy-2-deoxyguanosine Antibody Diluent (13 mL), part #6649.
- 1 bottle Wash Buffer Concentrate, 10X (50 mL), part #6650.
- 1 bottle TMB Substrate (13 mL), part #6651.
- 1 bottle Stop Solution (13 mL), part #6652.
- Kit manual.

3. REQUIRED MATERIALS

- Colorimetric plate reader capable of measuring absorbance at 450 nm.
- Distilled or deionized water (diH₂O).
- Adjustable pipettes, repeater pipet, and disposable tips.
- Materials used for sample preparation (see Section 8).

4. STORAGE

The reagents in this kit are stored at two different temperatures. Immediately open kit and store accordingly:

- Store the 8-hydroxy-2-deoxyguanosine Standard (part #6646) at -20°C. For optimum storage, it may be aliquoted into smaller portions and then stored frozen (10 μ L of Standard can prepare a triplicate standard curve). Avoid repeated freeze/thaw cycles.
- Store the rest of the kit at 4°C.
- Kit expires 1 year after receipt.

5. SDS

Safety data sheets are available online at www.immunochemistry.com or by calling 1-800-829-3194 or 952-888-8788.

6. PRECAUTIONS

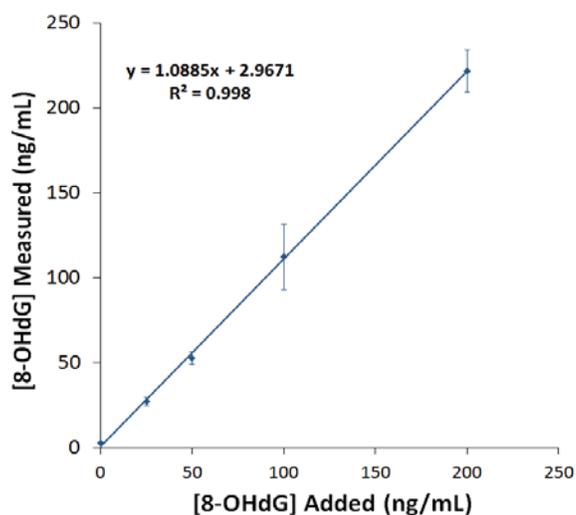
- As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The entire manual should be read and understood before attempting to use the product.
- This kit may not perform as described if any reagent or procedure is replaced or modified.
- Individual components may contain preservatives. Wear gloves while performing the assay. Please follow proper disposal procedures.
- **The Stop Solution (#6652) contains 1M hydrochloric acid (HCl). It is a strong acid solution and should be treated like any other laboratory acid.**

7. DETECTION EQUIPMENT

The assay can be analyzed with a colorimetric plate reader:

- A 96-well plate reader capable of reading optical absorption at 450 nm.
- Software to collect raw optical density (OD) readings from the plate reader and transform the data into a 4-parameter logistic curve (4PLC) fit. Contact your plate reader manufacturer for details.

Figure 2: Recovery of 8-hydroxy-2-deoxyguanosine from urine



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

8. SAMPLE PREPARATION

Please read this section thoroughly before beginning the assay. Proper sample storage and preparation are essential for consistent and accurate results.

- Caution should be taken during sample processing to avoid inadvertent oxidation of undamaged DNA.
- Prepare at least 180 μL of each diluted sample to analyze it in triplicate (approximately 50 μL /well).
- All samples must be free of organic solvents prior to testing.
- Samples that cannot be assayed immediately should be stored as indicated below.
- All suggested dilutions are simply recommended as a starting point, and it may be necessary to adjust the dilutions based on experimental results.

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 2). Urinary concentrations of 8-OHdG 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 0.2 μm 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 suggested starting dilution prior to testing. For example: put 9 μL of sample into 171 μL of Sample and Standard Diluent.

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 pathways of free versus DNA-incorporated 8-OHdG should be considered in the experimental design. If measuring DNA-incorporated 8-OHdG in plasma, it is possible 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 suggested starting dilution prior to testing. For example: put 9 μL of sample into 171 μL of Sample and Standard Diluent.

Culture Media Samples:

Storage: Collect culture media samples and store at -80°C .

Dilution: Fetal bovine serum contains 8-OHdG, therefore assays should either be performed in serum-free medium or PBS; these samples may be assayed directly. If the 8-OHdG concentration is high enough to dilute the sample 1:10 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 that was used for the experiment. This will ensure that the matrix for the standards is comparable to the samples. We recommend running a standard curve first to ensure that the assay will perform in a particular culture medium.

Cell Lysates:

Storage: Collect lysates using established methods and store at -80°C until use.

Usage:

1. Purify DNA using a commercially available extraction kit.
2. Digest DNA using nuclease P1 (such as Sigma N8630 or equivalent) following the manufacturer's instructions.
3. Adjust pH to 7.5-8.5 using 1M Tris.
4. Add 1 unit of alkaline phosphatase per 100 μg of DNA.
5. Incubate at 37°C for 30 minutes.
6. Boil for 10 minutes.
7. Place on ice until use.

Tissue Samples:

Storage: Snap-freeze tissue samples in liquid nitrogen immediately after collection. Store at -80°C until use.

Usage:

1. Prepare homogenization buffer: 0.1 M phosphate buffer, pH 7.4, containing 1 mM EDTA.
2. When ready to use the samples, thaw and add 5 mL of homogenization buffer per gram of tissue.
3. Homogenize the sample using either a Polytron-type homogenizer or a sonicator.
4. Centrifuge at 1,000 x g for 10 minutes.
5. Purify the supernatant using a commercially available DNA extraction kit.
6. Digest DNA using nuclease P1 (such as Sigma N8630 or equivalent) following the manufacturer's instructions.
7. Adjust the pH to 7.5-8.5 using 1 M Tris.
8. Add 1 unit of alkaline phosphatase per 100 μg of DNA.
9. Incubate at 37°C for 30 minutes.
10. Boil for 10 minutes.
11. Place on ice until use.

Saliva:

Storage: Saliva samples should be stored at -80°C immediately after collection. Samples may be assayed directly after appropriate dilution.

Dilution: Saliva samples may be diluted 1:8 (v:v) in Sample and Standard Diluent as a suggested starting dilution. For example: put 22.5 μL of sample into 157.5 μL of Sample and Standard Diluent.

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9. STANDARD (S1 - S8) & BLANK PREPARATION

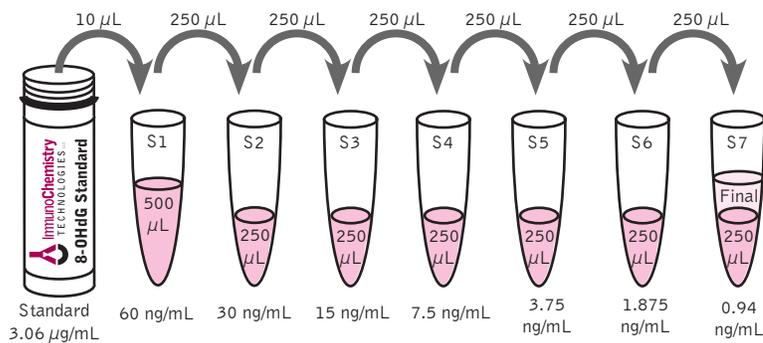
The 8-hydroxy-2-deoxyguanosine Standard at 3.06 $\mu\text{g/mL}$ (part #6646) should be aliquoted into smaller portions before use and frozen to ensure product integrity. Avoid freeze/thaw cycles.

10 μL of Standard is needed to prepare the standard curve in triplicate (Figure 3). Include a standard curve each time the assay is performed.

The Sample and Standard Diluent (part #6648) is supplied ready to use as a 1X solution. It is used to create the standard curve and dilute samples. It is also run in the assay as the Zero Standard (S8) and as the Blank to assess background noise. During the assay, the Zero Standard will receive 50 μL /well of the antibody preparation, while the Blank will receive 50 μL /well of the Antibody Diluent (Table 1).

- Before removing the cap, centrifuge the vial of the 8-hydroxy-2-deoxyguanosine Standard #6646 to ensure that all of the standard is collected and available for use.
- Label 8 polypropylene 1.5 mL tubes with one of the following standard values (Figure 3):
 - #S1 = 60 ng/mL
 - #S2 = 30 ng/mL
 - #S3 = 15 ng/mL
 - #S4 = 7.5 ng/mL
 - #S5 = 3.75 ng/mL
 - #S6 = 1.875 ng/mL
 - #S7 = 0.94 ng/mL
 - #S8 = 0 ng/mL = Zero Standard
- Add 500 μL of Sample and Standard Diluent to Tube #S1.
- Add 250 μL of Sample and Standard Diluent to the rest of the tubes (S2-S8).
- Set aside Tube #S8 (the Zero Standard), as it will not receive any of the standard.
- Add 10 μL of the 3.06 $\mu\text{g/mL}$ 8-hydroxy-2-deoxyguanosine Standard #6646 to Tube #S1 to yield a concentration of 60 ng/mL for the highest standard. Mix well.
- Transfer 250 μL from Tube #S1 to Tube #S2. Mix well.

Figure 3: Preparation of the 8-OHdG standards

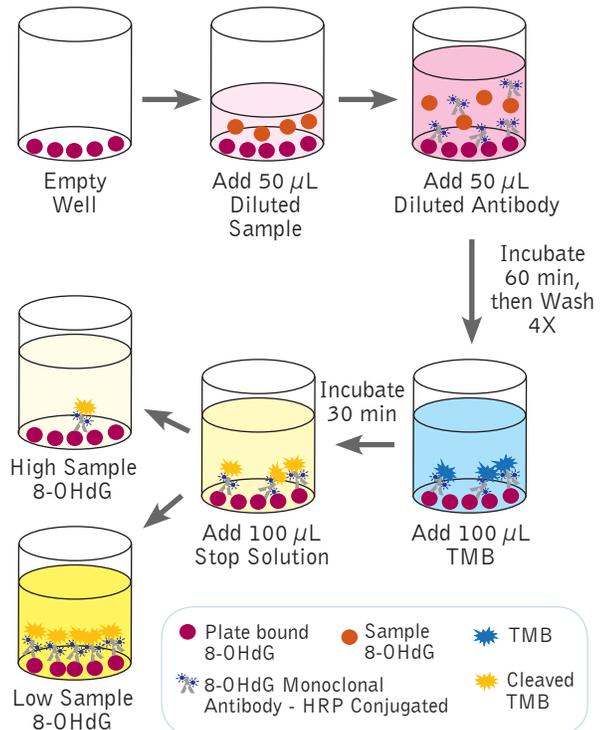


- Similarly, complete the dilution series to generate the remaining standards (250 μL from Tube #S2 to Tube #S7, mix well, etc.) up to and including Tube #S7. Tube #S7 is the low standard at 0.94 ng/mL.
- Do **NOT** add any standard to Tube #S8 as it is the Zero Standard (0 ng/mL). It only contains the Sample and Standard Diluent (Tube #S8 is not shown in Figure 3). During the assay, all the standards including the Zero Standard will receive 50 μL /well of the antibody preparation.
- Label another tube "Blank" and add approximately 180 μL of Sample and Standard Diluent. During the assay (Table 1), the Blank wells (containing 50 μL /well of the Sample and Standard Diluent) will receive the Antibody Diluent (50 μL /well); they will **NOT** receive the antibody preparation (Section 12). The Blank will be used to assess background noise (the Blank is not shown in Figure 3).

10. 1X WASH BUFFER PREPARATION

- Prepare 1X Wash Buffer by diluting the 10X Wash Buffer Concentrate (part #6650) 1:10 in distilled or deionized water. For example, if preparing 500 mL of 1X Wash Buffer, dilute 50 mL of 10X Wash Buffer Concentrate into 450 mL of distilled water.
- Mix well.
- Store reconstituted 1X Wash Buffer at 2-8°C for up to 1 month.
 - Take care to avoid contamination. Do not use 1X Wash Buffer if it becomes visibly contaminated during storage.

Figure 4. Assay Overview



11. HRP CONJUGATED MONOCLONAL ANTIBODY PREPARATION

1. Determine the amount of Antibody Preparation required. For every strip-well used (8-wells), prepare 0.5 mL of Antibody Preparation. 1 plate requires 6 mL.
2. Prepare Antibody Preparation by diluting the 8-OHdG HRP Conjugated Antibody (part #6647) 1:100 with 8-hydroxy-2-deoxyguanosine Antibody Diluent (part #6649). For example, if 6 mL of Antibody Preparation is required (1 whole plate), dilute 60 μ L of Antibody in 5.94 mL of 8-hydroxy-2-deoxyguanosine Antibody Diluent. Mix well.
3. Set aside approximately 180 μ L of the Antibody Diluent as this will be added to the Blank wells during the assay (50 μ L per Blank well) to assess background noise (Table 1).

12. ASSAY PROTOCOL

Tips:

- Allow the kit reagents to come to room temperature (RT) for 30 minutes.
- Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit (Section 8).
- Buffers may crystallize over time. Warm crystallized buffer until the salt crystals return to solution (ensure that components return to RT before use in the assay).
- ICT recommends that standards and samples be run in triplicate to accurately determine 8-OHdG concentrations.
- Use new pipette tips for each transfer to avoid cross-contamination.
- 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.
- Always add the Antibody Preparation **AFTER** the rest of the reagents, as this is a competitive assay.
- The well strips may be taped to the plate frame with lab tape as an extra precaution to prevent the plate strips from coming loose during the procedure.
- Use a new adhesive plate cover for each incubation step.
- Vigorous plate washing is essential.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.

Configure Plate Map:

- The 96-well plate included with this kit is supplied ready to use. It is not necessary to rinse the plate prior to adding the reagents.
- If all of the strips will not be used at one time, place the unused strips back in the plate bag and store at 4°C. Be sure the bag is sealed with the desiccant inside.
- For statistical purposes, ICT recommends assaying samples in triplicate.
- Record the position of the standards, blanks, and samples that will go in each well; see Figure 6 for a 96-well plate template.

Add Reagents (Table 1):

1. Add 50 μ L (in triplicate) of each of the following to the designated wells:
 - Prepared 8-hydroxy-2-deoxyguanosine Standards (Tubes #S1 through #S7).
 - Zero Standard (Tube #S8 containing just the Sample and Standard Diluent, which represents 0 ng/mL).
 - Blank (containing just the Standard and Sample Diluent, which will reveal background noise).
 - Samples (previously prepared; See Section 8: Sample Preparation).
2. Add 50 μ L of the diluted 8-OHdG Antibody Preparation (Section 11) to the wells containing the standards (#S1-S7), the Zero Standard (#S8), and the unknown samples. Do **NOT** add it to the Blank wells.
3. Add 50 μ L of the Antibody Diluent to the Blank wells only.

Table 1: Addition of Reagents

Well	Step 12.1		Step 12.2	Step 12.3	Total Volume Per well
	Standard or Sample Preparation	Standard and Sample Diluent	Antibody Preparation	Antibody Diluent	
Standard (S1-S7)	50 μ L	Included in Standard prep	50 μ L	Included in Ab. prep	100 μ L
Zero Standard (S8)	-	50 μ L	50 μ L	Included in Ab. prep	100 μ L
Blank	-	50 μ L	-	50 μ L	100 μ L
Unknown Samples	50 μ L	Included in Sample prep	50 μ L	Included in Ab. prep	100 μ L

Incubate the Reagents:

1. Cover plate with the plate cover and incubate 1 hour at room temperature (20-25°C).

Wash:

1. Carefully remove adhesive plate cover and discard it. Do **NOT** reuse the plate cover. Gently squeeze the long sides of the plate frame before washing to ensure all strips remain securely in the frame. Empty plate contents.
2. Use a multi-channel pipette to fill each well completely (300 μ L) with 1X Wash buffer, then empty plate contents.
3. Repeat procedure three additional times, for a total of **FOUR** washes.
4. Blot plate onto paper towels or other absorbent material.
 - Follow the same procedure when using an automated plate washer. Take care to avoid microbial contamination of equipment. Automated plate washers can easily become contaminated thereby causing assay variability.

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Incubate TMB Substrate and Stop the Reaction:

- Only remove the required amount of TMB Substrate (part #6651) and Stop Solution (part #6652) for the number of strips being used.
 - Do **NOT** use a glass pipette to measure the TMB Substrate.
 - Do **NOT** return leftover TMB Substrate to bottle.
 - Do **NOT** contaminate the unused TMB Substrate.
 - If the TMB Substrate is blue before use, **DO NOT USE IT**.
1. Add 100 μ L of TMB Substrate into each well.
 2. Cover carefully with a new plate cover. Do **NOT** reuse the plate cover from the first incubation step.
 3. Incubate at room temperature (20-25°C) in the dark for 30 minutes. During this incubation period, the enzymatic color reaction will develop; the substrate reaction yields a blue solution.
 4. Carefully remove the plate cover and discard.
 5. Add 100 μ L of Stop Solution into each well to stop the reaction.
 6. Tap plate gently to mix. The solution in the wells should change from blue to yellow.

Measure Absorbance:

1. Analyze the plate within 30 minutes of stopping the reaction.
2. Wipe the underside of plate wells with a lint-free tissue.
3. Measure the absorbance on a colorimetric ELISA plate reader set at 450 nm.

13. CALCULATION OF RESULTS

Many plate readers come with data reduction software that plot data automatically. When using such software, ensure that the following are performed by the software:

1. Average the triplicate optical density (OD) readings for each standard and sample.
2. Subtract the mean OD of the Zero Standard (S8).
3. Create a standard curve by reducing the data using the 4PLC fitting routine.
4. Multiply the concentrations obtained by the dilution factor to calculate sample values.
5. 8-OHdG concentrations are calculated from the data using the curve fitting routine supplied with the plate reader.

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

1. Calculate the average Net Optical Density (OD) bound for each standard and sample by subtracting the average Blank OD from the average OD bound.
2. Plot Net OD versus Concentration of 8-OHdG for the standards. Sample concentrations may be calculated off of Net OD values using the desired curve fitting.

3. Samples that read at concentrations outside of the standard curve range will need to be re-analyzed using a different dilution. Make sure to multiply sample concentrations calculated off the curve by the dilution factor used during sample preparation to get starting sample concentration.

14. ANALYTICAL PERFORMANCE CHARACTERISTICS

Assay Range: The assay range was determined to be 0.94 - 60 ng/mL.

Sensitivity: The sensitivity was determined to be 0.59 ng/mL

Precision:

Intra-Assay Precision (Within Run Precision): To determine intra-assay precision, 3 samples of known concentration were assayed 30 times on 1 plate. The intra-assay coefficient of variation was determined to be < 5%.

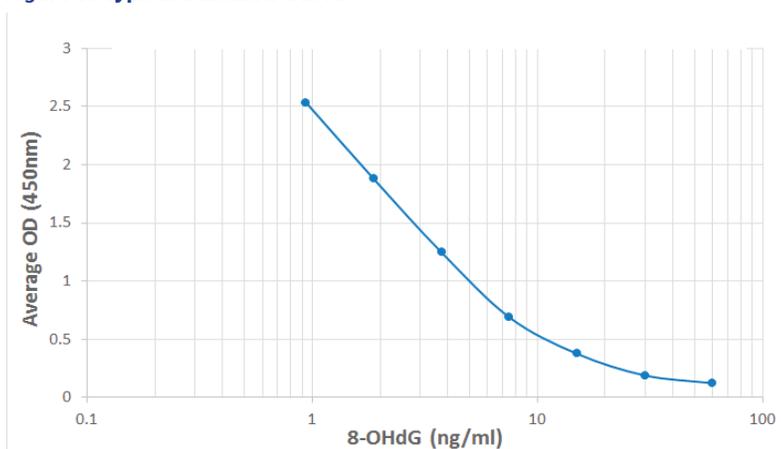
Inter-Assay Precision (Between Run Precision): To determine inter-assay precision, 3 samples of known concentration were assayed 30 times in 3 individual assays. The inter-assay coefficient of variation was determined to be < 5%.

Specificity:

Table 2: Specificity of the 8-OHdG Monoclonal Antibody

Compound	Cross-reactivity
8-hydroxy-2-deoxyguanosine	100 %
8-hydroxyguanosine	23 %
8-hydroxyguanine	23 %
Guanosine	< 0.01 %

Figure 5: Typical Standard Curve



*This standard curve was generated using the 8-OHdG ELISA Kit protocol.
This standard curve is for demonstration only.
A standard curve must be generated for each assay.*

15. ASSAY LIMITATIONS

- This assay has been validated for use with urine. Other sample types or matrices (e.g. tissue and cell extracts, cerebrospinal fluid, cell culture supernatant, etc.) may contain interfering factors that can compromise the performance of the assay, or produce inaccurate results.
- If an unknown sample generates results (OD value) that fall outside of the range of the standard curve, the samples should be concentrated or diluted (depending on whether sample gave a result below the lowest standard or above the highest standard, respectively) to bring the sample result within the range of the standard curve.
- 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.

16. TROUBLESHOOTING

Table 3: Troubleshooting

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

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Figure 6: 96-well Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

17. REFERENCES

1. Floyd, R.A. Role of oxygen free radicals in carcinogenesis and brain ischemia. *FASEB J.* 4, 2587-2597 (1990).
2. Spencer, J.P.E., Jenner, A., Chimel, K., et al. DNA strand breakage and base modification induced by hydrogen peroxide treatment of human respiratory tract epithelial cells. *FEBS Lett.* 374, 233-236 (1995).
3. Epe, B., Ballmaier, D., Roussyn, I., et al. DNA damage by peroxynitrite characterized with DNA repair enzymes. *Nucleic Acids Res.* 24, 4105-4110 (1996).
4. Beckman, K.B. and Ames, B.N. Oxidative decay of DNA. *J. Biol. Chem.* 272, 19633-19636 (1997).
5. Shen, J., Deininger, P., Hunt, J.D., et al. 8-hydroxy-2'-deoxyguanosine (8-OHdG) as a potential survival biomarker in patients with nonsmall-cell lung cancer. *Cancer.* 109, 574-580 (2007).
6. Kuo, H.-W., Chou, S.-Y., Hu, T.-W., et al. Urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) and genetic polymorphisms in breast cancer patients. *Mutat. Res.* 631, 62-68 (2007).
7. Endo, K., Miyashita, Y., Sasaki, H., et al. Probuco and atorvastatin decrease urinary 8-hydroxy-2'-deoxyguanosine in patients with diabetes and hypercholesterolemia. *J. Atheroscler. Thromb.* 13, 68-75 (2006).
8. Leinonen, J., Lehtimäki, T., Toyokuni, S., et al. New biomarker evidence of oxidative DNA damage in patients with non-insulin-dependent diabetes mellitus. *FEBS Lett.* 417, 150-152 (1997).
9. Lee, J., Lee, M., Kim, J.-U., et al. Carvedilol reduces plasma 8-hydroxy-2'-deoxyguanosine in mild to moderate hypertension. A pilot study. *Hypertension.* 45, 986-990 (2005).
10. Bogdanov, M.B., Beal, M.F., McCabe, D.R., et al. A carbon column-based liquid chromatography electrochemical approach to routine 8-hydroxy-2'-deoxyguanosine measurements in urine and other biologic matrices: A one-year evaluation of methods. *Free Radic. Biol. Med.* 27(5/6), 647-666 (1999).
11. Lin, H.-S., Jenner, A.M., Ong, C.N., et al. A high-throughput and sensitive methodology for the quantification of urinary 8-hydroxy-2'-deoxyguanosine: Measurement with gas chromatography-mass spectrometry after single solid-phase extraction. *Biochem. J.* 380, 541-548 (2004).

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*Thank you for using this kit!
If you have any questions, or would like to share your
data, please contact us at
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