Agrisera

# DNA Damage (8-OHdG) ELISA Kit

**Product# AS15 2887 (96-Well Kit)**

**Product# AS15 2887X5 (5 x 96-Well Kit)**

Colorimetric detection of 8-hydroxy-2-deoxy Guanosine

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# GENERAL INFORMATION

## Materials Supplied:

| Item | 96 wells Quantity/Size |
|---|---|
| 8-Hydroxy-2-Deoxy Guanosine: BSA Coated Plate | 1 Plate |
| 8-Hydroxy-2-Deoxy Guanosine standard | 1 vial/100µl |
| 8-Hydroxy-2-Deoxy Guanosine HRP Conjugated Monoclonal Antibody | 1 vial/75µl |
| Sample and Standard Diluent (Red) | 1 vial/50ml |
| 8-Hydroxy-2-Deoxy Guanosine Antibody Diluent (Blue) | 1 vial/13ml |
| Wash Buffer Concentrate (10X) | 1 vial/50ml |
| TMB Substrate | 1 vial 13ml |
| Stop Solution | 1 vial/13ml |
| Plate Cover | 2 |

If any of the items listed above are damaged or missing, please contact support@agrisera.com, or call +46 935 330 00. We cannot accept any returns without prior authorization.

**WARNING:** **Not for human or animal disease diagnosis or therapeutic drug use.**

## Precautions

**Please read these instructions carefully before beginning this assay.**

The reagents in this kit have been tested and formulated to work exclusively with Agrisera's ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

Agrisera suggests running all ELISA kits in triplicate, but replication must be defined by the user.

**For research use only. Not for human or diagnostic use.**

## If You Have Problems

**Technical Service Contact Information**


| | |
|---|---|
| **Phone:** | +46 935 330 00 |
| **Fax:** | +46 935 330 44 |
| **E-mail:** | support@agrisera.com |


In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

## Storage and Stability

This kit will perform as specified if the components are stored as directed and used before the expiration date indicated on the outside of the box.

All reagents are stable as supplied at 4°C, except the standard, 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 (10µl of Standard can prepare a triplicate standard curve).

## Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm.
2. Adjustable pipettes and a repeat pipettor.
3. Deionized or distilled water
4. Materials used for Sample Preparation (see page 9-11).

## Assay Precautions

- All ELISA reagents must be at room temperature before use.
- Vigorous plate washing is essential.
- Use new disposable pipette tips for each transfer to avoid cross-contamination.
- 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.
- Avoid microbial contamination of reagents and equipment. Automated plate washers can easily become contaminated thereby causing assay variability.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, do NOT use it.
- Individual components may contain preservatives. Wear gloves while performing the assay. Please follow proper disposal procedures.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in triplicate.
- Buffers may crystallize over time. Warm crystallized buffer until the salt crystals return to solution. Ensure that your components return to RT before use in the assay.

# INTRODUCTION

## Background

8-hydroxy-2-deoxy Guanosine (8-OHdG) is produced by the oxidative damage of DNA (see Figure 1, on page 7) 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]

## About This Assay

Agrisera's 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-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.

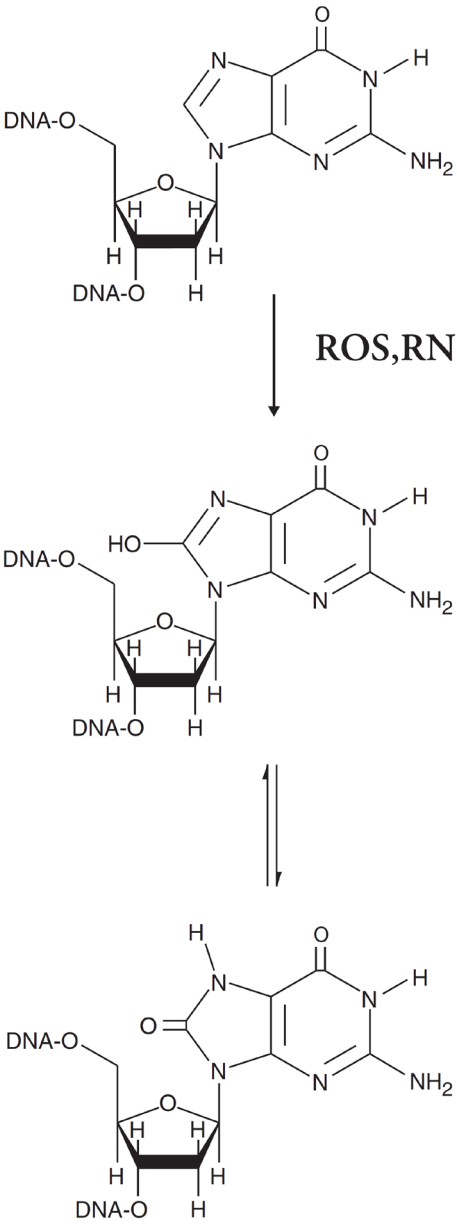



**Figure 1. Oxidation of Guanosine**

## Assay Overview

1. Prepare standard and samples in the Sample and Standard Diluent.
2. Add 50 µL of prepared standards and samples in triplicate to appropriate wells.
3. Add 50 µL of the diluted antibody preparation to the appropriate wells.
4. Cover plate with Plate Cover and incubate at room temperature (20-25°C) for 1 hour.
5. Wash plate 4 times with 1X Wash Buffer.
6. Add 100 µL of TMB Substrate to each well.
7. Cover plate and develop the plate in the dark at room temperature for 30 minutes.
8. Add 100 µL of Stop Solution to each well.
9. Measure absorbance on a plate reader at 450 nm.
10. Plot the standard curve and calculate sample concentrations.

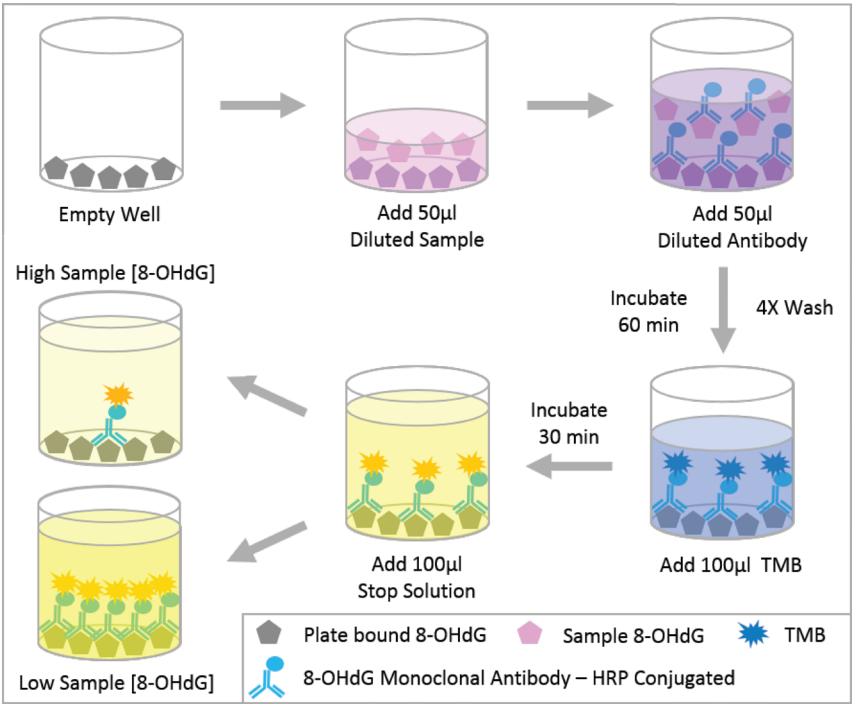



**Figure 2. Schematic of the 8-OHdG competitive ELISA**

# PRE-ASSAY PREPARATION

## Sample Preparation

Proper sample storage and preparation are essential for consistent and accurate results. Caution should be taken during sample work up, to avoid inadvertent oxidation of undamaged DNA. Please read this section thoroughly before beginning the assay.

*NOTE: Prepare at least 180µL of your diluted sample to permit assay in triplicate (approximately 50µL/ well).*

| 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. |

### 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 3). 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.

**Plasma/Serum**

The concentration of free 8-OHdG in plasma is very low relative to the level of DNAincorporated 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 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 starting dilution prior to testing.

**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 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.

**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 (Sigma N8630 or equivalent) 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.

**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 (Sigma N8630 or equivalent) 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.

**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.

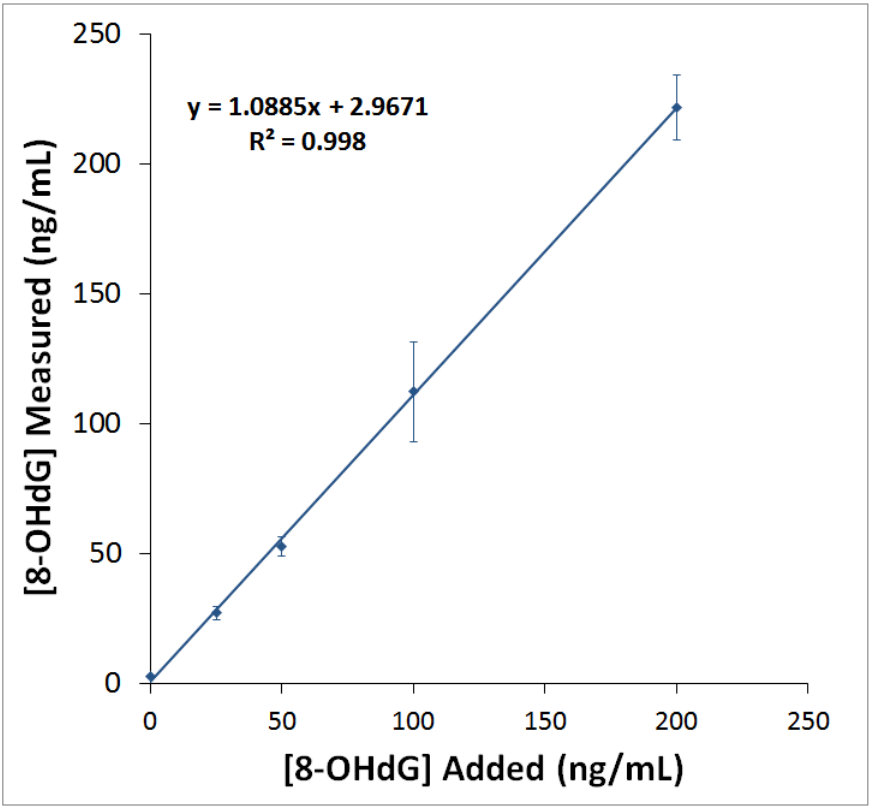



**Figure 3. Recovery of 8-hydroxy-2-deoxy Guanosine from urine**

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 unspiked urine. Error bars represent standard deviations obtained from multiple dilutions of each sample.

# Reagent Handling/ Preparation

**Standard Preparation (S1-S8)**

*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).*

1. Centrifuge the 8-hydroxy-2-deoxy Guanosine Standard vial before removing the cap. This process with assure that all of the standard is collected and available for use.
2. Label seven (7) polypropylene tubes, each with one of the following standard values: 60 ng/mL, 30 ng/mL, 15 ng/mL, 7.5 ng/mL, 3.75 ng/mL, 1.875 ng/mL and 0.94 ng/mL.
3. Add 500 µL of Sample and Standard Diluent to Tube #1.
4. Add 250 µL of Sample and Standard Diluent to Tube# 2, 3, 4, 5, 6 &7
5. Add 10 µL of the 3.06 µg/mL 8-hydroxy-2-deoxy Guanosine Standard to Tube #1 for a concentration of 60 ng/mL. Mix well.
6. Transfer 250 µL from Tube #1 to Tube #2. Mix well.
7. Similarily, complete the dilution series to generate the remaining standards (250 µL from Tube #2 to Tube #3, mix well, etc.) up to and including Tube #7.
8. Finally, add 250 µL Sample and Standard Diluent to another 1.5mL polypropylene tube (Tube #8), which is the zero standard (0 ng/mL).

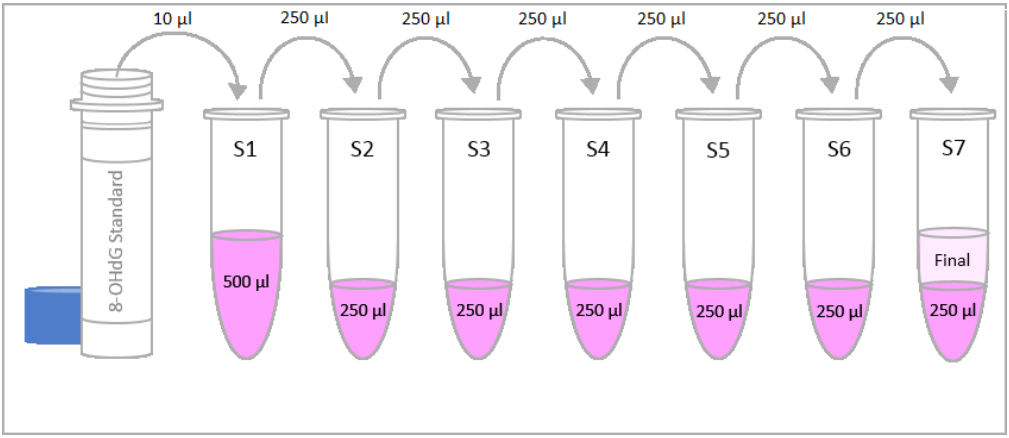



**Figure 4. Preparation of the 8-OHdG standards**

**1X Wash Buffer Preparation**

1. Prepare 1X Wash buffer by diluting 10X Wash Buffer in distilled or deionized water. For example, if preparing 500mL 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 2-8ºC for up to one (1) month. Do not use 1X Wash Buffer if it becomes visibly contaminated during storage.

**8-hydroxy-2-deoxy Guanosine: HRP Conjugate 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.

2. Prepare Antibody Preparation by diluting the 8-hydroxy-2-deoxy Guanosine: HRP Conjugate Antibody Concentrate 1:100 with 8-hydroxy-2-deoxy Guanosine 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-deoxy Guanosine Antibody Diluent. Mix well prior to use.

## Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all the strips at once, place the unused strips back in the plate packet and store at 2-4°C. Be sure the packet is sealed with the desiccant inside.*

For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 5, 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. We suggest you record the contents of each well on the template sheet provided (see page 26).

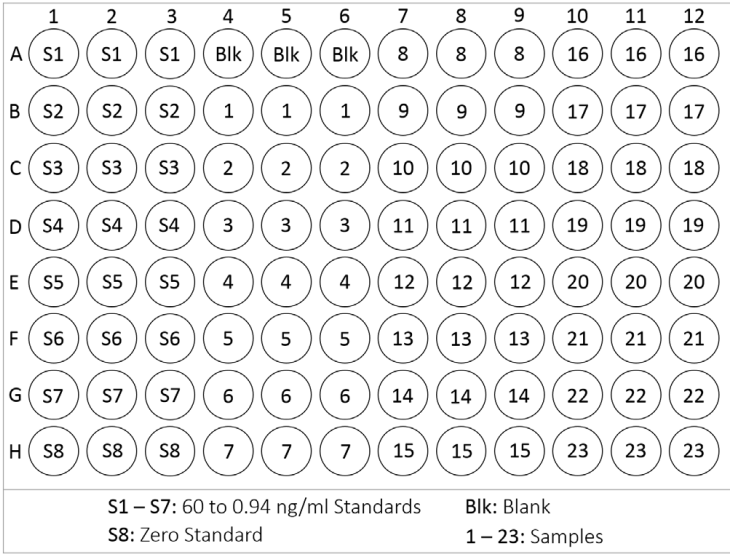



**Figure 5. Sample plate format**

# Performing the Assay

**Assay Hints**

- Use different tips to pipette the buffer, standard, sample, 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.
- Always add the Antibody Preparation after the rest of the reagents, as this is a competitive assay.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.

| Well | Standard OR Sample Preparation | Standard and Sample Diluent | Antibody Preparation | Antibody Diluent | Total Volume Per Well |
|---|---|---|---|---|---|
| Standard (S1-S7) | 50 µL | Included in Standard Prep | 50 µL | Included in Ab. Prep | 100 µL |
| Zero Standard (S8) | - | 50 µL | 50 µL | Included in Ab. Prep | 100 µL |
| Blank | - | 50 µL | - | 50 µL | 100 µL |
| Samples (1-23) | 50 µL | Included in Sample Prep | 50 µL | Included in Ab. Prep | 100 µL |

**Table 1: Pipetting Summary**

**Addition of the Reagents**

1. Add 50 µl (in triplicate) of each of the following to appropriate wells:

   • Prepared 8-hydroxy-2-deoxy Guanosine Standard (Tube #1 through 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, pages 9-11) into wells labelled 1-23

2. Add 50 µl of the previously diluted 8-hydroxy-2-deoxy Guanosine Antibody Preparation to each well, except the blank.

3. Add 50 µl of Standard and Sample Diluent and 50 µl of Antibody Diluent into wells labelled as the blank.

**Incubate the Plate**

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

**Plate Washing**

1. 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.
2. Empty plate contents. Use a multi-channel pipette to fill each well completely (300 µl) with 1X Wash buffer, then empty plate contents. Repeat procedure three additional times, for a total of FOUR washes. Blot plate onto paper towels or other absorbent material.

*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.*

**TMB Substrate Incubation and Reaction Stop**

- 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.

1. Add 100 µL of TMB Substrate into each well.
2. Cover carefully with the second provided plate cover.
3. Allow the enzymatic color reaction to develop at room temperature (20-25ºC) in the dark for 30 minutes. The substrate reaction yields a blue solution.
4. 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.

**Absorbance Measurement**

*Note: Evaluate the plate within 30 minutes of stopping the reaction.*

1. Wipe underside of wells with a lint-free tissue.
2. Measure the absorbance on an ELISA plate reader set at 450 nm.

# ANALYSIS

## Data Analysis Methods

This kit can be analyzed using any of the following methods:

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

**B.** Agrisera has a computer spreadsheet available for data anaylsis. Please visit our website (www.agrisera.com) to obtain a free copy of this convenient data analysis tool. Instructions on how to use this analysis tool are detailed in the excel file.

**C.** 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.

# Performance Characteristics

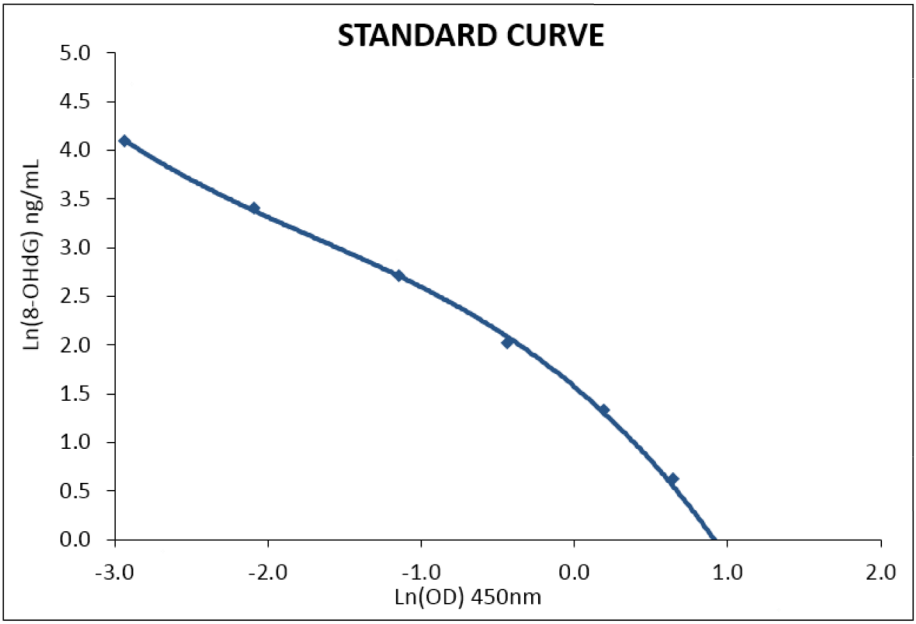



**Figure 6. Typical standard curve**
*NOTE: This typical standard curve was generated using the 8-OHdG ELISA Kit Protocol. This standard curve is for demonstration only. A standard curve must be generated for each assay.*

**Assay Range:** 0.94-60 ng/mL

**Sensitivity:**

- The sensitivity of the DNA Damage ELISA kit has been determined to be 0.59 ng/mL.

**Precision:**

1. Intra-Assay Precision (Within Run Precision)

- To determine Intra-Assay Precision, three samples of known concen tration were assayed thirty times on one plate. The intra-assay coefficient of variation of the DNA Damage ELISA has been determined to be <5%.

2. Inter-Assay Precision (Between Run Precision)

- To determine Inter-Assay Precision, three samples of known concentration were assayed thirty times in three individual assays. The inter-assay coefficient of variation of the DNA Damage ELISA has been determined to be <5%.

**Specificity:**

| Compound | Cross-reactivity |
|---|---|
| 8-hydroxy-2-deoxy Guanosine | 100% |
| 8-hydroxy Guanosine | 23% |
| 8-hydroxy Guanine | 23% |
| Guanosine | <0.01% |

**Table 2. Specificity of the 8-hydroxy-2-deoxy Guanosine Monoclonal Antibody**

**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 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.
- 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

# RESOURCES

## Troubleshooting

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

## 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. Probucol 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).

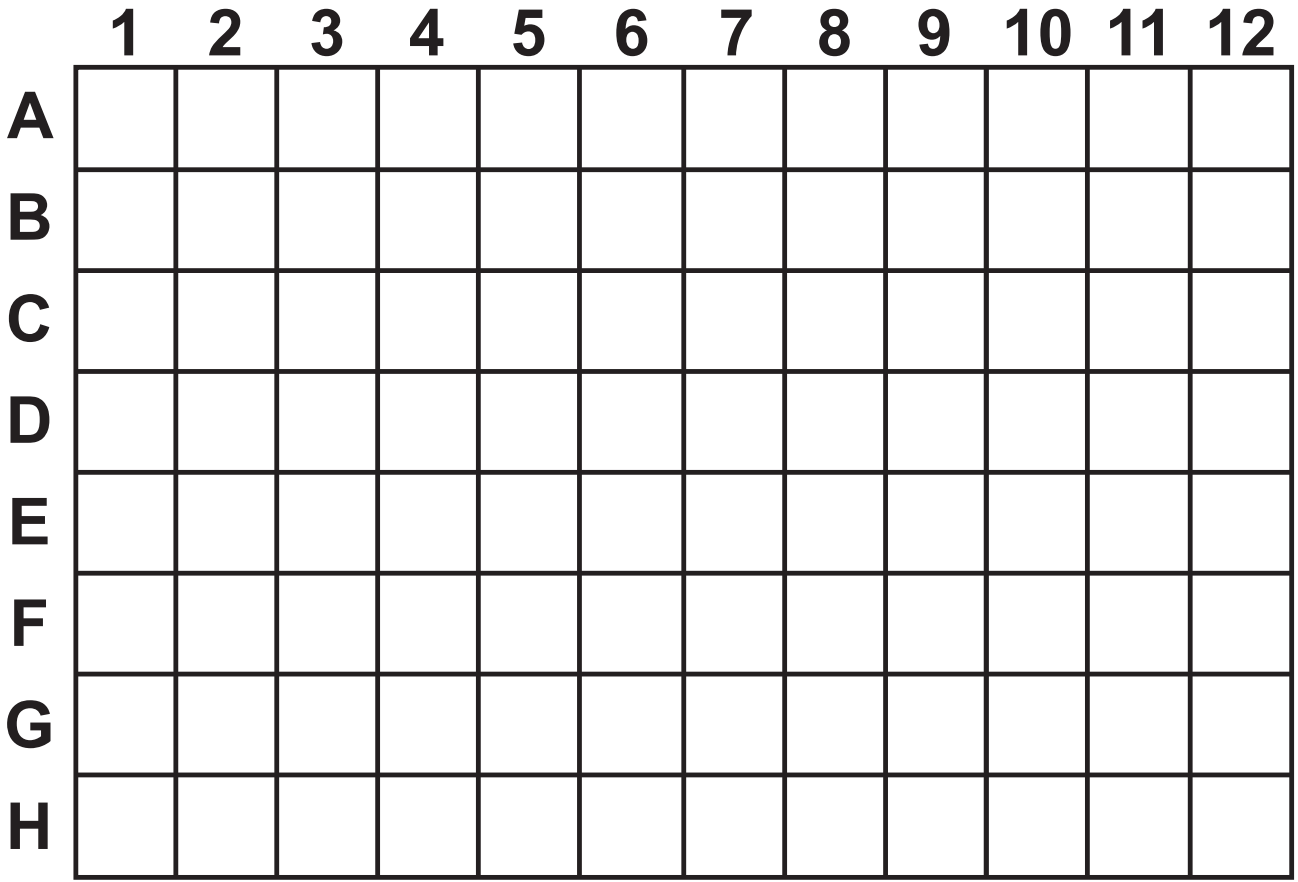
## Warranty and Limitation of Remedy

Agrisera makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Agrisera **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Agrisera will carry out its delivery obligations with due care and skill. Thus, in no event will Agrisera have any obligation or liability, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Agrisera is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Agrisera, its directors or its employees.

Buyer's **exclusive remedy** and Agrisera's sole liability hereunder shall be limited to a refund of the purchase price, or at Agrisera's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Agrisera within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

**For further details, please refer to our Warranty and Refund Policy located on our website and in our catalog.**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# NOTES: