MODIFIED STANDARD RANGE

コスモ・バイオ株式会社

※本プロトコールは参考用の資料になります。商品ご購入の際は必ず商品に添付されている資料をご参照ください。

Enzo Life Sciences

Enabling Discovery in Life Sciences®

DNA Damage EIA kit

For the detection and quantitation of 8-hydroxy-2'-deoxyguanosine in urine, serum, and saliva samples.

Catalog Number: ADI-EKS-350

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.

ALEXIS assay designs BIOMOL Stressgen

www.enzolifesciences.com

TABLE OF CONTENTS

A. INTRODUCTION

ASSAY DESIGN

The DNA Damage ELISA (enzyme-linked immunosorbent assay) is a fast and sensitive competitive immunoassay for the detection and quantitation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in urine, serum, and saliva samples. 8-OHdG has become a frequently used biomarker of oxidative DNA damage and oxidative stress. Measurement of urinary 8-OHdG may be useful as an indicator of oxidative damage.

The DNA Damage ELISA uses an 8-OHdG monoclonal antibody to bind, in a competitive manner, 8-OHdG in the sample, standard or pre-bound to the wells of the 96-well immunoassay plate. Anti-8-OHdG bound to 8-OHdG in the sample or standard are washed away while those captured by the immobilized 8-OHdG are detected with a secondary antibody: HRP conjugate. The assay is developed with tetramethylbenzidine substrate and the absorbance is measured in a microplate reader at 450nm. The intensity of the yellow color is inversely proportional to the concentration of 8-OHdG.

SCIENTIFIC OVERVIEW

Intracellular and extracellular free radical species can be potentially damaging to the living cell[1]. Intracellular free radical species are produced as a result of normal metabolism and extracellular forms are produced as a result of ultraviolet radiation or ionizing radiation[1]. Reactive oxygen species (ROS) are of particular interest in the research of oxidative damage and disease[1]. The various ROS include the highly reactive hydroxy radical ($^{\bullet}OH$), superoxide radical ($O_2^{\bullet -}$), hypochlorite ion ($OCl^{\bullet -}$) and non-radical hydrogen peroxide (H_2O_2)[1]. DNA, lipids, and proteins are cellular targets for oxidative damage by ROS and the order of preference for modification depends on location of ROS production, availability of metal ions, and the relative ability for the target to be oxidized[1]. Cells have acquired a number of defense mechanisms to cope with oxidative damage by ROS and other free radicals[1]. The simplest defense mechanisms involve Vitamin C and E intercepting free radicals, becoming radicals themselves and protecting cellular biomolecules from damage[1]. Complex defense mechanisms involve enzymes such as superoxide dismutase, catalase and glutathione peroxidase that have evolved to reduce ROS levels[1]. Low background levels of damage occur even in normal cells because ROS have a tendency to escape the defense mechanisms[1]. However, when the defense mechanisms cannot prevent the accumulation of ROS, then there is an increase in cellular damage[1].

INTRODUCTION

Modified lipids and proteins are removed via normal lipid-, protein- turnover mechanisms[1]. However, modified DNA cannot be replaced and has to be repaired[1]. Numerous DNA repair mechanisms have evolved in the cell and have become the focus of research in many disease states[2]. Removal of DNA damage and restoration of the continuity of the DNA duplex response, activation of the DNA damage checkpoint, which stops cell cycle and prevents the transmission of damaged chromosomes, changes in the transcriptional response of the cell and apoptosis are some of the important DNA damage response reactions[2].

8-hydroxy-2'-deoxyguanosine (8-OHdG) is a modified nucleoside base, which is the most commonly studied and detected by-product of DNA damage that is excreted in the urine upon DNA repair[3]. Urinary 8-OHdG and its analogs, 8-hydroxyguanosine and 8-hydroxyguanine, are linked to many degenerative diseases[3]. The association of ROS and the use of 8-OHdG as a biomarker of oxidative stress have been investigated in many diseases, including bladder and prostate cancer [3,4], cystic fibrosis [5], atopic dermatitis[6] and rheumatoid arthritis[7]. Parkinson's disease, Alzheimer's disease and Huntington's disease are neurodegenerative diseases that are thought to be caused by exposure to neurotoxins in people with a genetic predisposition for these diseases [8,9]. Oxidative stress is associated with the pathogenesis of these diseases and elevated levels of DNA damage have been measured in a wide range of neurological conditions [8,9].

ASSAY PROCEDURE SUMMARY

1. Bring to room temperature: **8-OHdG Immunoassay Plate, 20X Wash Buffer, Sample Diluent, Antibody Diluent, HRP Conjugate Diluent, TMB Substrate** and **Stop Solution 2.**
2. Prepare **8-OHdG Standard** and samples in **Sample Diluent**.
3. Add 50µL prepared standards and samples in duplicate to wells of **8-OHdG Immunoassay Plate**.
4. Add 50µL diluted **Anti-8-OHdG** to each well, except the blank. Cover immunoassay plate.
5. Incubate plate at room temperature for 1 hour.
6. Wash wells 6X using 300µL/well of 1X Wash Buffer.
7. Add 100µL diluted **Anti-Mouse IgG: HRP Conjugate** to each well, except the blank. Cover immunoassay plate.
8. Incubate plate at room temperature for 1 hour.
9. Wash wells 6X using 300µL/well of 1X Wash Buffer.
10. Add 100µL **TMB Substrate** to each well.
11. Incubate at room temperature for 15 minutes (preferably in the dark).
12. Add 100µL **Stop Solution 2** to each well.
13. Measure absorbance at 450nm.
14. Plot the 8-OHdG standard curve and calculate 8-OHdG sample concentrations. Use the Calculations Worksheet available on the website for calculation of results.

B. MATERIALS

PRECAUTIONS

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

- The activity of the **Anti-Mouse IgG horseradish peroxidase conjugate** *(part# 80-1515)* is affected by nucleophiles such as azide, cyanide and hydroxylamine.

- The **Stop Solution 2** *(part# 80-0377)* is a 1 Normal (1N) solution of hydrochloric acid. This solution is corrosive; please use caution when handling.

Please read the complete kit insert before performing this assay.

MATERIALS PROVIDED

The DNA Damage ELISA kit contains the following components in sufficient quantities for 96 wells. These reagents are sufficient to assay one standard curve and 39 samples in duplicate or two standard curves and 30 samples in duplicate.

PART #	COMPONENT	SIZE	DESCRIPTION
80-1512	8-OHdG Immunoassay Plate	96 well plate	12 x 8 removable strips and plate frame. Pre-coated plate with 8-OHdG: BSA conjugate
80-1513	8-OHdG Standard	25µL	10µg/mL stock solution of 8-hydroxy-2'deoxyguanosine
80-1510	Sample Diluent	50mL	Buffer to dilute standards and samples
80-1287	20X Wash Buffer	100mL	Concentrated solution of buffer and surfactant
80-1514	Anti-8-OHdG	25µL	Monoclonal antibody specific for 8-OHdG
80-1511	Antibody Diluent	6mL	Buffer for dilution of Anti-8-OHdG
80-1515	Anti-Mouse IgG: HRP Conjugate	25µL	Anti-Mouse IgG conjugated to horseradish peroxidase
80-1508	HRP Conjugate Diluent	11mL	Buffer for dilution of Anti-Mouse IgG: HRP Conjugate
80-0350	TMB Substrate	10mL	Stabilized tetramethylbenzidine substrate
80-0377	Stop Solution 2	10mL	Acid stop solution to stop color reaction

STORAGE OF MATERIALS

All reagents are stable as supplied at 4°C, except the **8-OHdG Standard**, which should be stored at -20°C. For optimum storage, the **8-OHdG Standard** should be aliquotted into smaller portions and stored at -20°C. Avoid repeated freeze/thaw cycles.

Unused wells of the **8-OHdG Immunoassay Plate** should be resealed with desiccant in the foil pouch provided and stored at 4°C until the kits expiry date.

MATERIALS REQUIRED BUT NOT PROVIDED

- Deionized or distilled water
- Precision pipettors capable of accurately delivering 1 to 1000µL
- Disposable pipette tips
- 5, 10, 25mL pipettes for reagent preparation
- 1L graduated cylinder
- Squirt bottle, manifold dispenser, or automated microtiter plate washer
- Disposable polypropylene tubes
- Microtiter plate reader capable of measuring absorbance at 450nm
- Adhesive plate sealers or plastic wrap

C. PERFORMING THE ASSAY

CRITICAL ASSAY PARAMETERS AND NOTES

- The DNA Damage ELISA kit contains a pre-coated microtiter plate (**8-OHdG Immunoassay Plate**) with removable wells to allow assaying on separate occasions.
- Run both standards and samples in duplicate.
- Include a standard curve each time the assay is performed.
- The following kit components should be brought to room temperature prior to use: **8-OHdG Immunoassay Plate**, **Sample Diluent**, **Wash Buffer**, **Antibody Diluent, HRP Conjugate Diluent, TMB Substrate, Stop Solution 2.**
- Absorbance is a function of the incubation time. Therefore, prior to starting the assay it is recommended that all reagents are ready to use and all required strip-wells are secured in the microtiter frame. This will ensure equal elapsed time for each pipetting step, without interruption.
- For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 15 minutes.
- Mix all reagents and samples gently, yet thoroughly, prior to use. Avoid foaming of reagents.
- To avoid cross contamination, change disposable pipette tips between the addition of each standard, sample, and reagent. Use separate reagent troughs/reservoirs for each reagent.
- This assay requires pipetting of small volumes. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated.
- Consistent, thorough washing of each well is critical. If using an automatic washer, check washing head before use. If washing manually, ensure all wells are completely filled at each wash. Air bubbles should be avoided.
- Exercise appropriate laboratory safety precautions when performing this assay.
- In this protocol, room temperature refers to 20-28°C. The room temperature should remain within this range throughout the assay.

NOTE: *The components in each kit lot # have been quality assured and warranted in this specific combination only; please do not mix them with components from other kit lot #s.*

SAMPLE PREPARATION

*** Caution should be taken during sample work up, to avoid inadvertent oxidation of undamaged DNA.**

1. EXTRACTION OF SAMPLES

Urine Samples

Fresh clear urine samples can be diluted in **Sample Diluent** and used directly in the assay. Samples containing precipitate should be centrifuged at 2,000 x *g* for 10 minutes, or filtered using a 0.45µm filter, prior to use in the assay.

Urine samples can be frozen at –70°C and assayed at a later date. Filter samples using a 0.45µm filter and add suitable antibiotics, such as gentamicin at 30µg/mL, or 0.05% sodium azide, prior to storing at –70°C.

Serum Samples

a) Collect whole blood using established methods.
b) Allow samples to clot at room temperature for 30 minutes.
c) Centrifuge at 2700 x *g* for 10 minutes, taking precautions to avoid hemolysis.
d) Remove serum. Transfer the serum to a labeled polypropylene tube. The serum collected is now ready for analysis using the DNA Damage ELISA kit.
e) Alternatively, the serum sample can be frozen at ≤-20°C and assayed at a later date. It is recommended that the serum be aliquotted to convenient volumes prior to storing at ≤-20°C to avoid multiple freeze thaw cycles.

Saliva Samples

Collect sample in centrifuge tube. To clarify, freeze sample at -70°C for 1 hour. Thaw sample on ice, and centrifuge at 2,000 x g for 10 minutes. Transfer clarified supernatant to clean tube for use in the assay. Alternatively, the clarified saliva samples can be frozen at ≤ -20°C and assayed at a later date. It is recommended that the saliva sample be aliquoted to convenient volumes prior to storing at ≤ -20°C to avoid multiple freeze thaw cycles.

2. DILUTION OF SAMPLES

Samples should be prepared as described above. Dilute prepared urine samples 1:20 (v/v) as a suggested starting dilution in **Sample Diluent**. Serum samples may be diluted 1:20 (v/v) appropriately in **Sample Diluent** as a suggested starting dilution. Dilute prepared saliva samples 1:8 (v/v) as a suggested starting dilution in **Sample Diluent**. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

a) Dilute prepared samples (i.e. urine, serum, saliva) in **Sample Diluent**. Prepare at least 150µL of diluted sample to permit assaying in duplicate.
b) Mix thoroughly.
c) Samples are now ready to be used in the Assay Procedure (see page 13). Samples may be left at room temperature while Reagents are being prepared (see page 10).

REAGENT PREPARATION

NOTE: *All reagents should be freshly prepared prior to use. Once prepared, reagents should be kept at room temperature for the duration of the assay.*

NOTE*: The preparation of the reagents is based on using the complete 1 X 96 well assay, unless otherwise noted. If only a portion of the immunoassay plate is to be used, please store all components as previously described (see page 7).*

1. TEMPERATURE OF REAGENTS

Bring the following reagents to room temperature prior to use:

- **8-OHdG Immunoassay Plate** *(Part#: 80-1512)*
- **Sample Diluent** *(Part#: 80-1510)*
- **Wash Buffer** *(Part#: 80-1287)*
- **Antibody Diluent** *(Part#: 80-1511)*
- **HRP Conjugate Diluent** *(Part#: 80-1508)*
- **TMB Substrate** *(Part#: 80-0350)*
- **Stop Solution 2** *(Part#: 80-0377)*

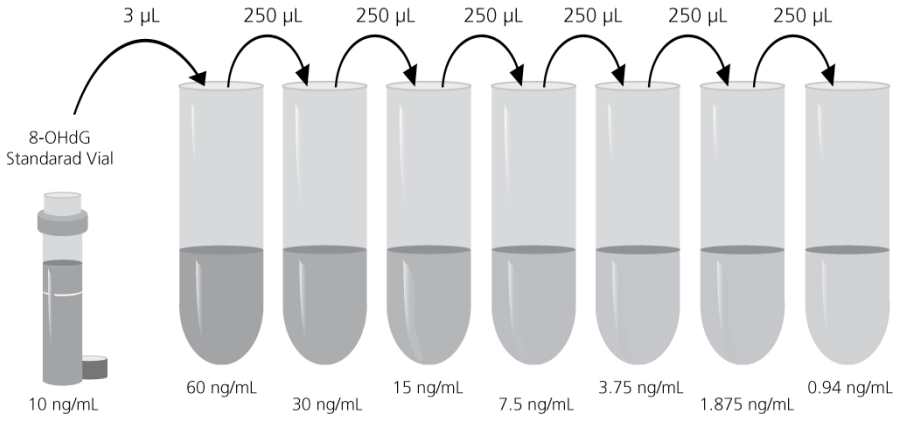
2. 8-OHdG STANDARD *(Part#: 80-1513)*

***NOTE**: The 8-OHdG Standard will withstand two freeze/thaw cycles to allow preparation of a second standard curve. However, to ensure product integrity, it is suggested that the 8-OHdG Standard be aliquotted into smaller portions and any remaining 8-OHdG Standard be discarded after the second use. Avoid repeated freeze/thaw cycles.*

The **8-OHdG Standard** is used to generate a standard curve with 7 points, ranging from 0.94 - 60ng/mL.

a) Centrifuge the **8-OHdG Standard** vial before removing the cap. This process will assure that all of the standard is collected and available for use.
b) Label seven (7) polypropylene tubes, each with one of the following standard values: 60ng/mL, 30ng/mL, 15ng/mL, 7.5ng/mL, 3.75ng/mL, 1.875ng/mL, 0.94ng/mL.
c) Add 500µL of **Sample Diluent** to Tube #1.

   Add 250µL of **Sample Diluent** to Tube #2, 3, 4, 5, 6, and 7.
d) Add 3µL of the **8-OHdG Standard** stock solution (10µg/mL) to Tube #1.
e) Mix thoroughly.
f) Transfer 250µL from Tube#1 to Tube #2.
g) Mix thoroughly.
h) Similarly, complete the dilution series to generate the remaining standards (250µL from Tube #2 to Tube #3, mix thoroughly, etc) up to and including Tube #7.
i) Finally, add 250µL Sample Diluent to another 1.5mL polypropylene tube (Tube # 8), which is the zero standard (0ng/mL).



3. WASH BUFFER *(Part#: 80-1287)*

   a) Bring the **20X Wash Buffer** to room temperature and swirl gently to dissolve any crystals that may have formed from storage.
   b) Dilute the 100mL of **20X Wash Buffer** with 1900mL of deionized or distilled water. Once diluted, the 1X Wash Buffer is stable at room temperature for up to 4 weeks. For longer-term storage, the Wash Buffer should be stored at 4˚C.

***NOTE**: 100mL of **20X Wash Buffer** has been provided in this kit, which is sufficient for the preparation of 2L of 1X Wash Buffer. The minimum required volume of 1X Wash Buffer is 350mL (if the complete plate is used at once). However additional 1X Wash Buffer is supplied to allow for multiple assays or alternative washing techniques.*

4. ANTI-8-OHdG *(Part#: 80-1514)*

   a) Centrifuge the vial before removing the cap to ensure maximum product recovery.
   b) Dilute 20µL of **Anti-8-OHdG** in 5mL of **Antibody Diluent** in a polypropylene tube. If only using a portion of the plate, dilute only what is needed for number of wells used.
   c) Mix gently by inversion.
   d) Reagent is now ready to be used in the Assay Procedure (see page 13).
   e) Do not re-use or store any remaining diluted **Anti-8-OHdG.**

5. ANTI-MOUSE IgG: HRP CONJUGATE *(Part#: 80-1515)*

   a) Centrifuge the vial before removing the cap to ensure maximum product recovery.
   b) Dilute 22µL of the **Anti-Mouse: HRP Conjugate** in 11mL of the **HRP Conjugate Diluent** in a polypropylene tube. If only using a portion of the plate, dilute only what is needed for the number of wells used.
   c) Mix gently by inversion.
   d) Reagent is now ready to be used in the Assay Procedure (see page 14).
   e) Do not re-use or store any remaining diluted **Anti-Mouse IgG: HRP Conjugate**.

ASSAY PROCEDURE

1. DETERMINE THE REQUIRED NUMBER OF WELLS
   a) Refer to the 8-OHdG Plate Template on page 21 to determine the number of wells to be used.
   b) Remove the **8-OHdG Immunoassay Plate** from the packaging and note the color of the desiccant pack. Silica beads should be blue. Pink beads indicate that moisture is present and the performance of the plate may be compromised.
   c) If less than 96 pre-coated microtiter wells are needed, remove the excess wells from the frame and return them to the foil pouch.
   d) Reseal the pouch containing the unused wells and store at 4°C.

2. ADDITION OF STANDARDS, SAMPLES AND ANTI-8-OHdG ANTIBODY
   a) Add 50µL (in duplicate) of each of the following to appropriate wells:
      - Prepared **8-OHdG Standard** (Tube#1 through Tube #7)
      - Zero Standard (**Sample Diluent**, which represents 0ng/mL)
      - Samples (previously prepared - see Sample Preparation, page 9)
   b) Add 50µL of the previously diluted **Anti-8-OHdG** to each well, except the blank.
   c) Cover wells with an adhesive plate sealer or plastic wrap and incubate at room temperature for 1 hour.

***NOTE**: For each step in the procedure, total dispensing time for the addition of the reagents and samples to the assay plate should not exceed 15 minutes.*

3. WASHING
   a) Aspirate liquid from all wells.
   b) Add 300µL of 1X Wash Buffer to all wells, using a multi-channel pipette, manifold dispenser, automated microplate washer, or a squirt bottle.
   c) Repeat the aspirating and washing 5 more times, for a total of 6 washes.
   d) After the 6th addition of 1X Wash Buffer, aspirate the liquid from all wells. Invert the plate and carefully pat dry on clean paper towels.

4. ADDITION OF ANTI-MOUSE IgG: HRP CONJUGATE
(previously diluted, see page 12)

a) Add 100µL of the previously diluted **Anti-Mouse IgG: HRP Conjugate** to each well, except the blank.
b) Cover wells with a fresh adhesive plate sealer (or plastic wrap) and incubate at room temperature for 1 hour.
c) Wash plate as described in Step #3.

5. ADDITION OF TMB SUBSTRATE AND STOP SOLUTION

a) Add 100µL of the **TMB Substrate** to the wells. Color development should be visible within 1 minute of addition to the plate.
b) Incubate the plate at room temperature for 15 minutes (preferably in the dark).
c) Add 100µL of the **Stop Solution 2** to the wells in the same order that the **TMB Substrate** was added.

6. MEASURING ABSORBANCE

a) Set up the microplate reader according to the manufacturer's instructions.
b) Set wavelength at 450nm.
c) Measure the absorbance. If the plate cannot be read immediately, it should be covered and kept at room temperature. The absorbance should be read within 30 minutes of adding the **Stop Solution 2**.

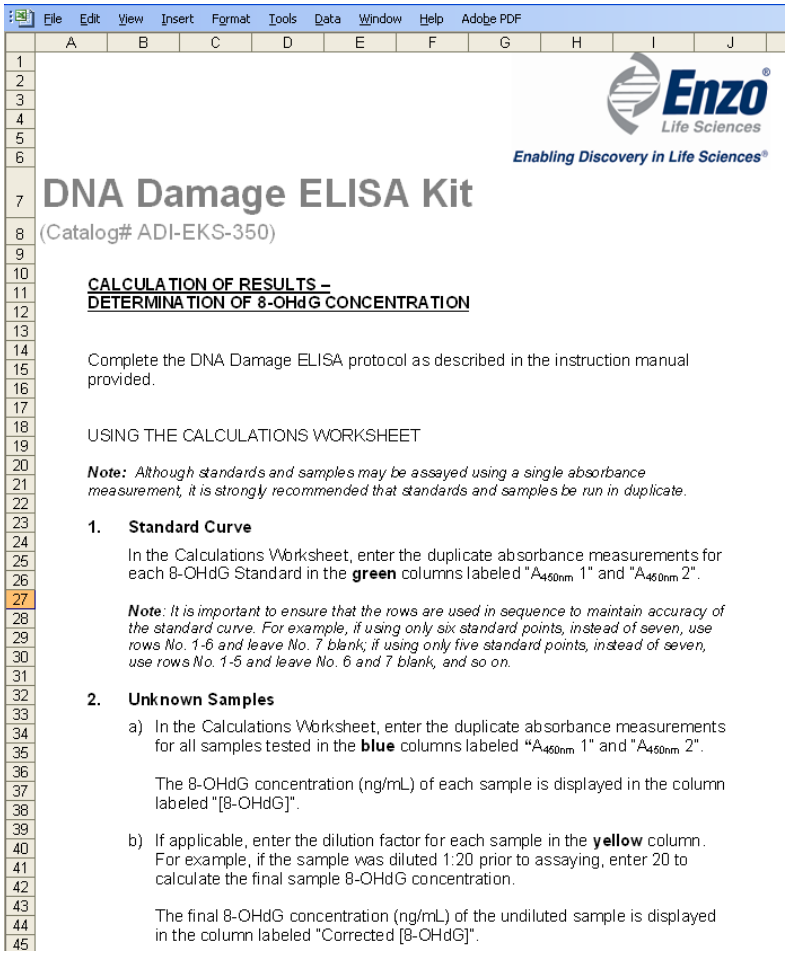
CALCULATION OF RESULTS - DETERMINATION OF 8-OHdG CONCENTRATIONS

1. To determine 8-OHdG assay results, use the Calculations Worksheet provided on the website: http://www.assaydesigns.com/ (MS Excel is required).

2. Follow the steps on the instructions page for using the Calculations Worksheet.

Print Screen of Instructions Page

(for illustration only)



File Edit View Insert Format Tools Data Window Help Adobe PDF

Enzo Life Sciences

Enabling Discovery in Life Sciences®

DNA Damage ELISA Kit

(Catalog# ADI-EKS-350)

CALCULATION OF RESULTS – DETERMINATION OF 8-OHdG CONCENTRATION

Complete the DNA Damage ELISA protocol as described in the instruction manual provided.

USING THE CALCULATIONS WORKSHEET

Note: *Although standards and samples may be assayed using a single absorbance measurement, it is strongly recommended that standards and samples be run in duplicate.*

1. **Standard Curve**

   In the Calculations Worksheet, enter the duplicate absorbance measurements for each 8-OHdG Standard in the **green** columns labeled "A_{450nm} 1" and "A_{450nm} 2".

   Note*: It is important to ensure that the rows are used in sequence to maintain accuracy of the standard curve. For example, if using only six standard points, instead of seven, use rows No. 1-6 and leave No. 7 blank; if using only five standard points, instead of seven, use rows No. 1-5 and leave No. 6 and 7 blank, and so on.*

2. **Unknown Samples**

   a) In the Calculations Worksheet, enter the duplicate absorbance measurements for all samples tested in the **blue** columns labeled "A_{450nm} 1" and "A_{450nm} 2".

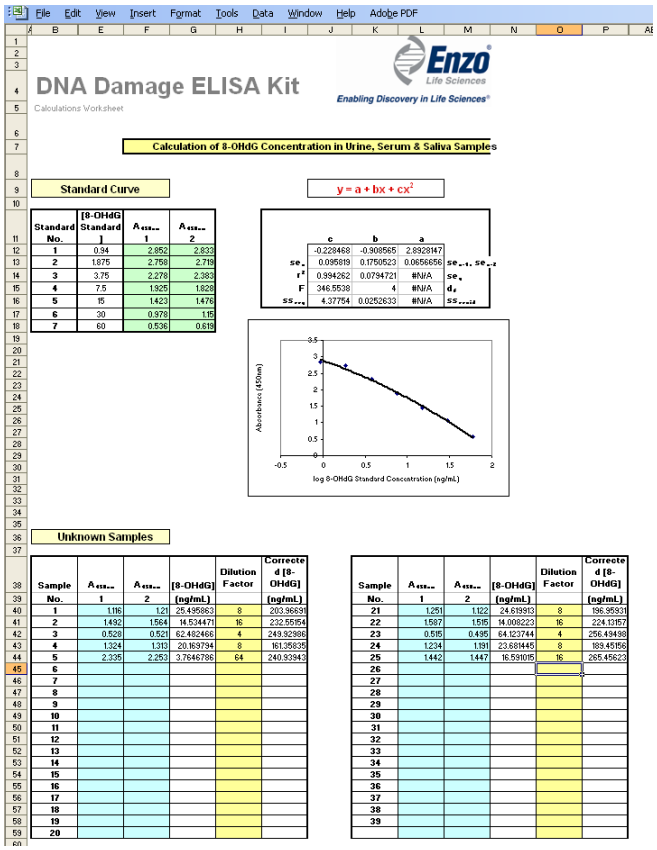
      The 8-OHdG concentration (ng/mL) of each sample is displayed in the column labeled "[8-OHdG]".

   b) If applicable, enter the dilution factor for each sample in the **yellow** column. For example, if the sample was diluted 1:20 prior to assaying, enter 20 to calculate the final sample 8-OHdG concentration.

      The final 8-OHdG concentration (ng/mL) of the undiluted sample is displayed in the column labeled "Corrected [8-OHdG]".

Print Screen of Calculations Worksheet

(for illustration only)



ALTERNATIVE METHOD FOR CALCULATING RESULTS

If the Calculations Worksheet cannot be accessed for calculating results, use the following steps.

1. Calculate the average of the duplicate absorbance measurements for each standard and sample.

2. Calculate the average of the duplicate absorbance measurements for the blank.

3. Subtract the average value obtained in Step#2 (blank) from the values obtained in Step#1 (standards and samples).

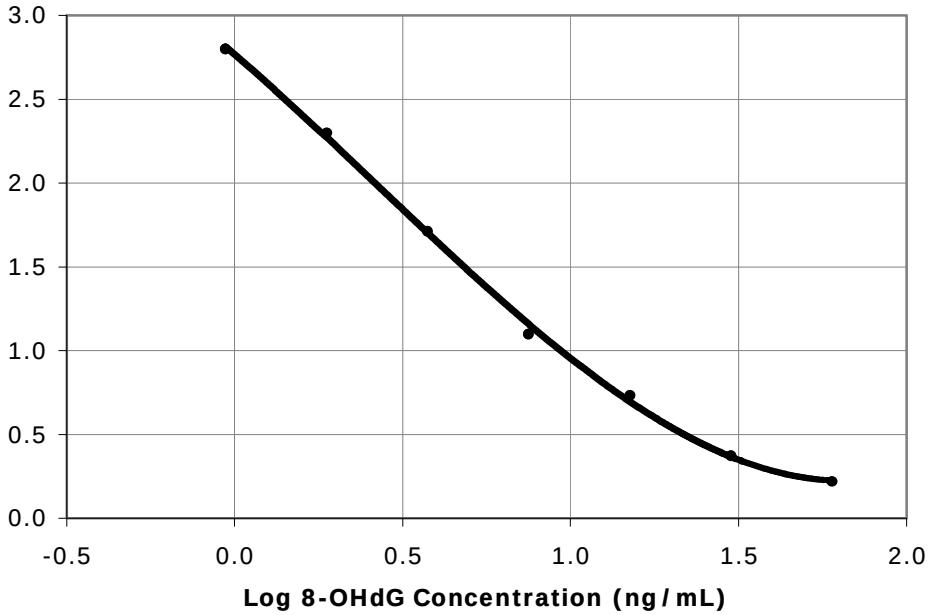
4. To generate the standard curve, plot the log of the 8-OHdG Standard concentrations (ng/mL) on the X-axis, and the absorbance measurements for the corresponding 8-OHdG standards on the Y-axis.

5. The standard curve is a 2nd order polynomial function represented by the equation: $y = a + bx + cx^2$, where y is the average absorbance measurement of the sample, x is the log of 8-OHdG concentration in ng/mL and a, b and c are coefficients.
   Calculate the 8-OHdG sample concentrations either by using the polynomial equation above or by interpolating the sample concentrations from the standard curve.

6. Multiply by the dilution factor for the final sample 8-OHdG concentration. For example, if the sample was diluted 1:20 prior to assaying, the value generated from the polynomial equation or the standard curve must be multiplied by 20 to calculate the final sample 8-OHdG concentration.

D. ASSAY PERFORMANCE CHARACTERISTICS

TYPICAL 8-OHdG STANDARD CURVE



PERFORMANCE CHARACTERISTICS

1. SENSITIVITY

   The sensitivity of The DNA Damage ELISA kit has been determined to be 0.59 ng/mL.
   The standard curve has a range of 0.94 - 60ng/mL.

2. PRECISION

   a) Intra-Assay Precision (Within Run Precision)

      To determine Intra-Assay Precision, three samples of known concentration were assayed thirty times on one plate.
      The Intra-Assay Coefficient of variation of the DNA Damage ELISA has been determined to be <10%.

   b) 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 <10%.

3. SPECIFICITY

The DNA Damage ELISA also detects 8-hydroxyguanosine (product of oxidative RNA damage) and 8-hydroxyguanine (product of oxidative DNA damage by hydroxyl radicals).

Other related compounds were identified, and these cross-reactants were diluted in sample diluent and assayed at concentrations of 100x, 10x, 1x, 0.1x, and 0.01x of high standard concentration. Cross-reactivity was calculated from ED50 of the cross-reactant divided by the ED50 of the standard curve. Where ED50's could not be obtained for a cross-reactant, it was assumed that cross-reactivity was less than 0.016% (lowest 8-OHdG standard divided by the highest cross-reactant concentration).

Cross Reactant	Cross-reactivity
Guanosine	< 0.016%
8-Bromoguanosine	< 0.016%
2'-Deoxyinosine	< 0.016%
8-Mercaptoguanosine	3.5%
N^2-Methylguanosine	< 0.016%

LIMITATIONS OF THE ASSAY

- 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.
- Although this assay has been validated for use with urine and serum samples, some samples may contain higher levels of interfering factors that can produce anomalous 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.

E. REFERENCES

REFERENCES

1. Evans, M. D., Dizdaroglu, M., and Cooke, M. S. (2004) *Mutat. Res.* **567**: 1-61.
2. Sancar, A., Lindsey-Boltz, L. A., Unsal-Kacmaz, K., and Linn, S. (2004) *Annu. Rev. Biochem.* **73**: 39-85.
3. Chiou, C. C., Chang, P. Y., Chan, E. C., Wu, T. L., Tsao, K. C., and Wu, J. T. (2003) *Clin. Chim. Acta.* **334**: 87-94.
4. Trzeciak, A. R., Nyaga, S. G., Jaruga, P., Lohani, A., Dizdaroglu, M., and Evans, M. K. (2004) *Carcinogenesis* **25:** 1359-1370.
5. Brown, R. K., McBurney, A., Lunec, J., and Kelly, F. J. (1995) *Free Radic. Biol. Med.* **18**: 801-806.
6. Tsuboi, H., Kouda, K., Takeuchi, H., Takigawa, M., Masamoto, Y., Takeuchi, M., and Ochi, H. (1998) *Br. J. Dermatol.* **138**: 1033-1035.
7. Rall, L. C., Roubenoff, R., Meydani, S. N., Han, S. N., and Meydani, M. (2000) *J. Nutr. Biochem.* **11:** 581-584.
8. Lezza, A. M., Mecocci, P., Cormio, A., Beal, M. F., Cherubini, A., Cantatore, P., Senin, U., and Gadaleta, M. N. (1999) *FASEB J.* **13**: 1083-1088.
9. Alam, Z. I., Jenner, A., Daniel, S. E., Lees, A. J., Cairns, N., Marsden, C. D., Jenner, P., and Halliwell, B. (1997) *J. Neurochem.* **69**: 1196-1203.

F. APPENDICES

APPENDIX –8-OHdG Immunoassay Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	7.5 ng/mL	0 ng/mL									
B	Blank	7.5ng/mL	0 ng/mL									
C	60 ng/mL	3.75 ng/mL										
D	60 ng/mL	3.75 ng/mL										
E	30 ng/mL	1.875 ng/mL										
F	30 ng/mL	1.875 ng/mL										
G	15 ng/mL	0.94 ng/mL										
H	15 ng/mL	0.94 ng/mL										

REFERENCE

1. Bring to room temperature: **8-OHdG Immunoassay Plate**, **20X Wash Buffer**, **Sample Diluent**, **Antibody Diluent**, **HRP Conjugate Diluent**, **TMB Substrate** and **Stop Solution 2**.
2. Prepare **8-OHdG Standard** and samples in **Sample Diluent**.
3. Add 50µL prepared standards and samples in duplicate to wells of **8-OHdG Immunoassay Plate**.
4. Add 50µL diluted **Anti-8-OHdG** to each well, except the blank. Cover immunoassay plate.
5. Incubate plate at room temperature for 1 hour.
6. Wash wells 6X using 300µL/well of 1X Wash Buffer.
7. Add 100µL diluted **Anti-Mouse IgG: HRP Conjugate** to each well, except the blank. Cover immunoassay plate.
8. Incubate plate at room temperature for1 hour.
9. Wash wells 6X using 300µL/well of 1X Wash Buffer.
10. Add 100µL **TMB Substrate** to each well.
11. Incubate at room temperature for 15 minutes (preferably in the dark).
12. Add 100µL **Stop Solution 2** to each well.
13. Measure absorbance at 450nm.
14. Plot the 8-OHdG standard curve and calculate 8-OHdG sample concentrations. Use the Calculations Worksheet on the website for calculation of results.

Enzo Life Sciences

Enabling Discovery in Life Sciences®

Use of Product

This product contains research chemicals. As such, they should be used and handled only by or under the supervision of technically qualified individuals. This product is not intended for diagnostic or human use.

Warranty

Enzo Life Sciences International, Inc. makes no warranty of any kind, expressed or implied, which extends beyond the description of the product in this brochure, except that the material will meet our specifications at the time of delivery. Enzo Life Sciences International, Inc. makes no guarantee of results and assumes no liability for injuries, damages or penalties resulting from product use, since the conditions of handling and use are beyond our control.

www.enzolifesciences.com

Enabling Discovery in Life Science®

North/South America
ENZO LIFE SCIENCES INT'L, INC.
5120 Butler Pike
Plymouth Meeting, PA 19462-1202/USA
Tel. 1-800-942-0430/(610)941-0430
Fax (610) 941-9252
info-usa@enzolifesciences.com

Germany
ENZO LIFE SCIENCES GmbH
Marie-Curie-Strasse 8
DE-79539 Lorrach / Germany
Tel. +49/0 7621 5500 526
Toll Free 0800 664 9518
Fax +49/0 7621 5500 527
info-de@enzolifesciences.com

UK & Ireland
ENZO LIFE SCIENCES (UK) LTD.
Palatine House
Matford Court
Exeter EX2 8NL / UK
Tel. 0845 601 1488 (UK customers)
Tel. +44/0 1392 825900 (overseas)
Fax +44/0 1392 825910
info-uk@enzolifesciences.com

Switzerland & Rest of Europe
ENZO LIFE SCIENCES AG
Industriestrasse 17, Postfach
CH-4415 Lausen / Switzerland
Tel. +41/0 61 926 89 89
Fax +41/0 61 926 89 79
Info-ch@enzolifesciences.com

Benelux
ENZO LIFE SCIENCES BVBA
Melkerijweg 3
BE-2240 Zandhoven / Belgium
Tel. +32/0 3 466 04 20
Fax +32/0 3 466 04 29
info-be@enzolifesciences.com

France
ENZO LIFE SCIENCES
c/o Covalab s.a.s.
13, Avenue Albert Einstein
FR-69100 Villeurbanne / France
Tel. +33 472 440 655
Fax +33 437 484 239
Info-fr@enzolifesciences.com

ALEXIS assay designs BIOMOL Stressgen
www.enzolifesciences.com

August 3, 2010