NWLSS™
8-OHdG ELISA

High Sensitivity
For Plasma, Tissue & Cell Derived Samples

Product NWK-8OHDG02
For Research Use Only

Simple assay kit for measurement of 8-hydroxydeoxyguanosine (8-OHDG) in samples expected to contain lower amounts of analyte such as plasma, tissue homogenates and cell lysates.

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References (Continued):

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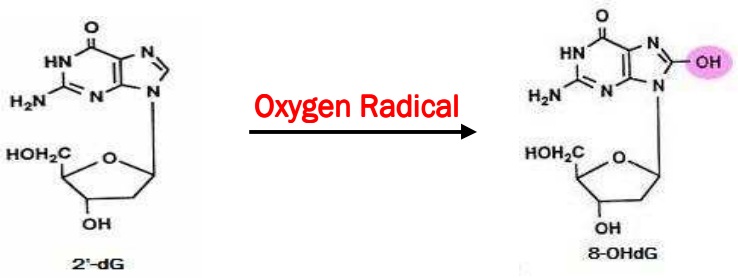
9. Tadashi Matsubasa, Takako Uchino, Shinnyo Karashima, Yuichi Kondo, Kenichi Maruyama, Masako Tanimura and Fumio Endo (2002) *Free Radical Research 36(2)* pp189193; Oxidative Stress in very low Birth Weight Infants As Measured by Urinary 8OHdG.

Statement of Limited Warranty:

Northwest Life Science Specialties, LLC (NWLSS) makes no guarantee of any kind, expressed or implied, that extends beyond the description of the material in this kit, except that they will meet our specifications at the time of delivery. Customer's remedy and NWLSS' sole liability is limited to, at NWLSS' option, refund of the purchase price, or the replacement of material not meeting our specification. By acceptance of our product, customer assumes all liability and will indemnify and hold NWLSS harmless for the consequence of this product's use or misuse by the customer, its employees, or others. Refund or replacement is conditioned of customer notifying NWLSS within twenty-one (21) days of the receipt of product. Failure to give notice within 21 days shall constitute a waiver by the customer of all claims hereunder with respect to said product.

Introduction:

Oxidative stress is known to play an important role in the development of various diseases and aging process. Under conditions of oxidative stress, 8-hydroxy-2'-deoxyguanosine (8-OHdG) is formed when DNA is oxidatively damaged by reactive oxygen species (ROS). 8-OHdG is one of the most sensitive biomarkers for oxidative stress and can be detected in serum, urine and/or DNA isolated from cells and tissues in humans and animals. The figure below depicts the oxidation 2'-deoxyguanosine (2'dG) to form the 8-OHdG biomarker.



Intended Use:

The NWLSS™ 8-OHdG ELISA is intended for quantitative detection of the 8-hydroxyl 2' deoxyguanosine oxidative DNA adduct in biological samples including urine, plasma, serum, tissue and other biological samples.

Test Principle:

The NWLSS™ 8-OHdG ELISA assay uses a competitive format wherein a murine monoclonal antibody to 8-OHdG (*Primary Anti-8OHdG*) and sample or standard are added to a microtiter plate which has been precoated with 8-OHdG. Sample or calibrator 8-OHdG competes with plate-bound 8-OHdG for binding with the antibody. Accordingly, higher concentrations of sample or calibrator leads to reduced binding of the antibody to the 8OHdG coated plate. A subsequent wash step removes any free 8-OHdG/antibody adduct leaving stationary plate bound 8-OHdG complexed to antibody for later detection. Anti-murine antibody conjugated to horse radish peroxidase (*HRP-Conjugate*) is then added to the plate. HRP-conjugate binds to remaining murine anti-8-OHdG and unbound HRP-conjugate is removed in another wash step. Addition of 3,3',5,5'tetramethylbenzidine (*TMB Substrate*) results in blue color development proportional to the amount of anti 8OHdG antibody bound to the plate and inversely proportional to the concentration 8-OHdg in original samples or calibrators applied to the plate. The reaction is terminated by addition of phosphoric acid (*Stop Solution*) producing yellow color with measurable absorbance at 450 nm.

General Specifications:

Format::	1 X 96 wells	
Number of tests:	Triplicate =	24
	Duplicate =	40
Specificity:	8-Hydroxy 2' deoxyguanosine	
Sensitivity:	LLD = 0.125 ng/mL in sample applied to plate	

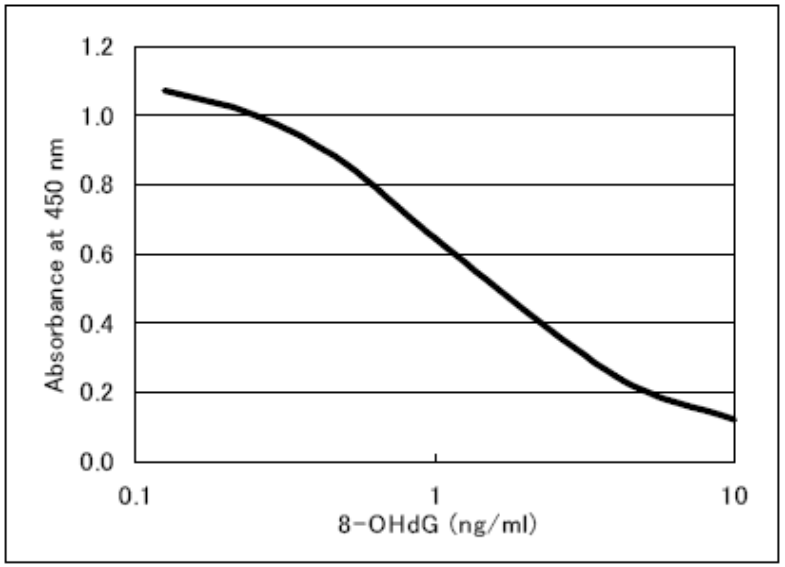
Kit Contents:

Microtiter Plate:	Precoated with 8OHdG	12 X 8 wells
8OHdG Standard:	6 levels Purified 8OHdG	6 X 1 mL Vials
Primary Antibody: Murine clone N45.1	Mu-Anti-8OHdG Monoclonal	1 vial (Lyoph.)
Primary Antibody Buffer:	Phosphate buffered saline	1 vial (6mL)
Secondary Antibody:	Anti-murine-HRP conjugate	1 vial (Lyoph.)
Secondary Antibody Buffer:	Phosphate buffered saline	1 vial (12mL)
TMB Substrate:	3,3',5,5'tetramethylbenzidine	1 vial (0.25mL)
Diluting Buffer:	H_2O_2/Citrate/PBS	1 vial (12mL)
Wash Buffer (5x):	Concentrated PBS	2 vials (26mL)
Stop Solution:	1M Phosphoric acid	1 vial (12mL)
Plate Seal		2 Sheets

Required Materials Not Provided:
Adjustable pipettes capable of transferring 50 µL to 200 µL volumes.
A multi-channel or repeater pipette (recommended).
Reagent Trays (if using multi-channel).
37°C Incubator
Distilled water

Required Instrumentation:
Microplate reader with 450nm capability.

Data Analysis:
Create a standard curve by plotting Absorbance vs. Concentration (log scale) for each standard level assayed. If available, set the plate reader to utilize 4-Parameter curve fit. An example standard curve is shown below.



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4. M.D.Evans, M.S.Cooke, I.D.Podmore, Q.Zheng, K.E.Herbert, and J.Lunec (1999) *Biochemical and Biophysical Research Communications 259 pp374378*; Discrepancies in the measurement of UVCinduced 8-oxo 2' deoxyguanosine: Implications for the analysis of oxidative DNA damage.

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Assay Protocol:

Standard Procedure for Microplate Assay

1. Bring all reagents to room temperature.

2. Remove appropriate number of wells for assay from foil pouch.

3. Construct an assay template to ensure proper sample addition.

4. Add 50µL of **Sample** or **Standard** to each well to be assayed.

5. Add 50µL **PBS** to blank wells.

6. Add 50µL **Reconstituted Primary Antibody** to each well to be assayed except blank wells.

7. Shake lightly side to side to ensure proper mixing.

8. Cover plate with adhesive strip, then incubate at 4°C overnight.

9. Empty contents of wells into sink and blot on paper towel to remove as much fluid as possible.

10. Wash plate 3 times as follows:
Add 250µL **Working Wash Buffer** per well
Shake plate slightly during soak period for best wash results.
Empty wash solution into sink by inversion then blot plate against clean paper towel to remove any remaining washing buffer.

11. Add 100µL of **Reconstituted Secondary Antibody** per well.

12. Shake lightly side to side to ensure to mixing.

13. Seal with adhesive strip then incubate at room temp for 1 hour.

14. Empty contents of wells into sink and blot on paper towel.

15. Wash the plate 3 times as in Step 10.

16. Add 100µL of **Working TMB Substrate** per well.

17. Shake lightly side to side to ensure proper mixing.

18. Incubate for 15 minutes, at room temp in the dark.

19. Add 100µL of **Stop Solution** per well.

20. Measure the absorbance at 450nm.

Warnings, Limitations, Precautions:

Incubation Temperature

Measured values can be affected by variations in incubation temperatures. Care should be exercised to assure uniform temperature during incubations, particularly during the Primary Antibody reaction period. It is recommended that incubation be performed in a humid environment (i.e. in an incubator with water present.

Sample pH

The optimal solution pH of sample mixed with primary antibody should be between 6.0 to 8.0. Samples with unusual pH can be diluted in PBS if pH is deemed to be an issue.

Possible edge effects

To minimize edge effects, ensure that plate is sealed properly and that incubation times are as uniform as possible. To maintain the most uniform temperature within the wells, it is recommended that any unused wells on a single strip be filled with an equal volume of water prior to incubation.

TMB Substrate:

Do not dilute TMB Substrate until just before needed. Keep it in the dark.

Blank wells:

Do not add Primary Antibody to Blank wells.

Storage Instructions:

Upon receipt, store the reagents at 2-8ºC. This kit is stable if unopened for 9 months from the date of manufacture. Do not use components beyond the expiration date printed on the kit box label.

Unused wells and opened reagents must be stored at 2-8ºC and should be used within two weeks after first opening.

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Assay Preparation

Plate Reader Setup

Wavelength:	450 nm
Mode:	Endpoint
Shaker:	On
Curve Fit:	4-Parameter Logistic

Reagent Preparation:

Bring all reagents, plate wells to be used samples and calibrators to room temperature (20-25°C) before use.

Day One (1):
Primary Antibody: (Red Cap and Label)
On Day One (1): Reconstitute the **Primary Antibody** with **Primary Antibody Buffer.**

Day Two (2)
Wash Buffer:
Prepare the necessary volume of wash buffer by mixing 1 part **5X Wash Buffer** with 4 parts distilled water. Label as **Working Wash Buffer.**

Secondary Antibody: (Blue Cap and Label)
Reconstitute the **Secondary Antibody** with **Secondary Antibody Buffer.**

TMB Substrate:
Just prior to use, prepare the necessary volume of TMB Substrate by mixing 1 part **TMB Substrate** with 100 parts **Dilution Buffer.** Label as **Working TMB Substrate.**

Sample Handling/Preparation:

The multi-disciplinary interest in measuring 8-OHdG has resulted in a myriad of sample types and experimental conditions and is beyond the scope of this product insert to describe sample processing in detail for each case. However, general guidelines are provided below for representative sample types.

Urine:
If urine samples are measured with this kit, then cloudy (opaque) samples should be centrifuged at ~2000-5,000g for 10-15 minutes. If urine is clear, no pretreatment is necessary and samples may be applied directly to the assay. It may be necessary to treat samples with PBS (pH 7.4) prior to assay to ensure an assay pH between 6.0 and 8.0 or if 8-OHdG exceeds the highest (10 ng/mL standard. For researchers who prefer same day results, we recommend using the our product number NWK-8OHDG01, Urinary 8-Hydroxydeoxyguanosine ELISA.

Sample Handling/Preparation (continued):

Serum or Plasma:
Blood samples must be separated immediately. To separate interfering substances from serum or plasma, ultrafiltration with a cut off molecular weight of 10,000 is necessary. Pretreat ultra filter according to manufacturer guidelines. To reduce deviation, it is recommended that samples be diluted more than 2X with PBS (pH 7.4) prior to ultra filtration.

Tissue or Cell Culture:
It is necessary to extract and digest sample DNA prior to assay. The method below is intended as a general guideline:

1. Required Reagents: Purified Water.
TE buffer: 1mM EDTA in 10mM Tris-HCl (pH8.0)

2. Homogenize the sample using a Dounce homogenizer, sonication or other suitable means.

3. Extract the DNA from homogenates using a commercially available DNA extraction kit following the manufacture's instruction or by other suitable laboratory protocol if available.

4. Calculate DNA Concentration and Purity:
DNA extract should be diluted to 20-50 µg/mL with TE buffer.
Measure the Absorbance of sample extracts at 260, 280 and 320nm.
ABS_{320} is used as a background measurement and an absorbance of 1.0 at 260nm is equal to 50 µg DNA/mL sample such that DNA concentration in a sample extract can be calculated using the equation:

$$[DNA\ (\mu g/mL)] = (ABS_{260} - ABS_{320}) * (50\ \mu g/mL) * dilution$$

The ABS_{260}/ABS_{280} ratio of pure DNA should be from 1.8 to 1.85.

5. *Enzymatic Digestion of DNA.*
Dissolve 200 µg of extracted DNA in 135 µL of water. Add 15 µL of 200mM sodium acetate and 6 units of nuclease P1 (15 µL of 1 mg/mL) to the DNA Solution. Incubate for 30 min to 1hr at 37°C under Argon.

Add 1M Tris-HCl buffer (15 µL, pH 7.4) and 2 unit of alkaline phosphatase (7 µL of 200 Unit/0.7mL), and incubate again for 30 min to 1hr at 37°C under Argon.

Remove enzymes and other macromolecules by filtering through Millipore Microcon YM-10 (catalog # 42407) at 14000 rpm for 10min.

DNA digest is ready for assay at 50µL per replicate. It is recommended that samples be assayed the same day enzymatic digestion is performed.