



DNA/RNA Oxidative Damage Express ELISA Kit

Item No. 502890

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd • Ann Arbor, MI • USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item Name	Quantity/Size	Storage
401155	DNA/RNA Oxidative Damage Express ELISA Antibody	1 vial/100 dtn	-20°C
401156	DNA/RNA Oxidative Damage Express ELISA AChE Tracer	1 vial/100 dtn	-20°C
401157	DNA/RNA Oxidative Damage Express ELISA Standard	1 vial	-20°C
400060	ELISA Buffer Concentrate (10X)	1 vial/10 ml	RT
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	RT
400035	Polysorbate 20	1 vial/3 ml	RT
400008/400009	Anti-Mouse IgG Coated Plate	1 plate	4°C
400012	96-Well Cover Sheet	1 cover	RT
400050	Ellman's Reagent	3 vials/100 dtn	-20°C
400040	ELISA Tracer Dye	1 vial	RT
400042	ELISA Antiserum Dye	1 vial	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.



WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's DNA/RNA Oxidative Damage Express ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

When compared to quantification by LC/MS or GC/MS, it is not uncommon for immunoassays to report higher analyte concentrations. While LC/MS or GC/MS analyses typically measure only a single compound, antibodies used in immunoassays sometimes recognize not only the target molecule, but also structurally related molecules, including biologically relevant metabolites. In many cases, measurement of both the parent molecule and metabolites is more representative of the overall biological response than is the measurement of a short-lived parent molecule. It is the responsibility of the researcher to understand the limits of both assay systems and to interpret their data accordingly.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888
Email: techserv@caymanchem.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 stored as directed at -20°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 405-420 nm
2. Adjustable pipettes; multichannel or repeating pipettor recommended
3. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
4. Materials used for Sample Preparation (see page 13)

Background

DNA and RNA are damaged by oxidation during aging and in a variety of disease states, including cancer.¹⁻³ Guanine is the base most prone to oxidation, and the repair processes initiated to correct the damage release multiple oxidized guanine/guanosine species into the urine, including 8-hydroxyguanosine (8-OHG), 8-hydroxy-2'-deoxyguanosine (8-OHdG), and 8-hydroxyguanine. Over 80% of DNA is transcribed into RNA, which is concentrated in the mitochondria.^{4,5} Mitochondria are the primary source of reactive oxygen species (ROS), therefore, the localization of RNA to the mitochondria increases the susceptibility of RNA to ROS-mediated attacks, which increases the potential for RNA-derived oxidized guanine/guanosine species, such as 8-OHG, to be present in various sample types. Oxidative damage to DNA can induce mutagenic properties and alter transcription factor binding, resulting in changes in epigenetic regulation.⁴ Urinary and blood levels of 8-OHdG are increased in patients with a variety of cardiovascular diseases, including heart failure and atherosclerosis.⁵ Brain levels of oxidized cytoplasmic RNA are increased in patients with Alzheimer's disease.^{4,5}

About This Assay

Cayman Chemical DNA/RNA Oxidative Damage Express Kit detects all three oxidized guanine/guanosine species; 8-OHdG from DNA, 8-OHG from RNA, and 8-hydroxyguanine from either DNA or RNA. Some commercial vendors offer immunoassays that detect only 8-OHdG, and not the other two molecules. The advantage of the Cayman kit is that it captures a more complete set of biologically relevant products of oxidative damage than do assays that are restricted to analysis of only 8-OHdG.

However, because Cayman's kit recognizes more than 8-OHdG, it is not valid to compare the results from the Cayman ELISA Kit to an LC/MS analysis of 8-OHdG. The ELISA value will always be significantly higher than LC/MS because the ELISA also detects 8-OHG and 8-hydroxyguanine.

This express assay has a range from 34.3-10,000 pg/ml and a lower limit of detection (LLOD) of 62 pg/ml.

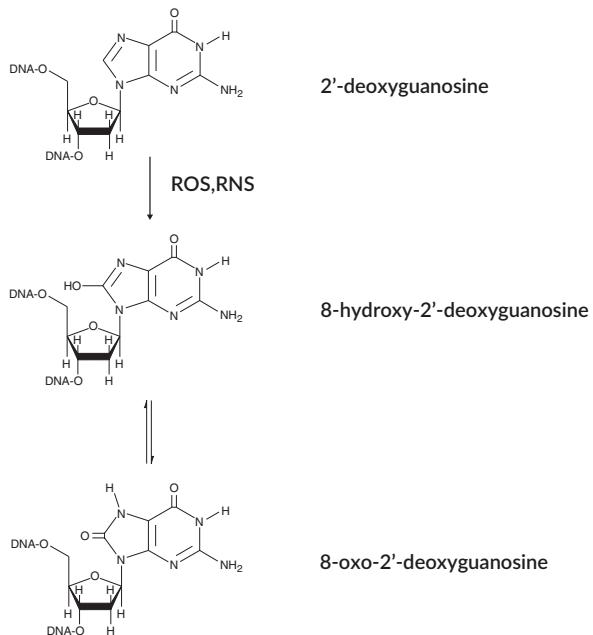


Figure 1. Oxidation of Guanosine

Principle Of This Assay

This assay is based on the competition between oxidatively damaged guanine/guanosine species and an 8-OHdG-acetylcholinesterase conjugate (DNA/RNA Oxidative Damage Express AChE Tracer) for a limited amount of DNA/RNA Oxidative Damage Express antibody. Because the amount of tracer is held constant while the concentration of oxidatively damaged guanine/guanosine species varies, the amount of tracer that is able to bind to the monoclonal antibody will be inversely proportional to the concentration of oxidatively damaged guanine/guanosine in the well. This antibody-oxidized guanine/guanosine complex binds to the anti-mouse IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and then Ellman's Reagent (which contains the substrate for AChE) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of DNA/RNA Oxidative Damage Express Tracer bound to the well, which is inversely proportional to the amount of free 8-OHdG present in the well during the incubation; or

$$\text{Absorbance} \propto [\text{Bound DNA/RNA Oxidative Damage Express Tracer}] \propto \frac{1}{[8\text{-OHdG}]}$$

A schematic of this process is shown in Figure 2, on page 10.

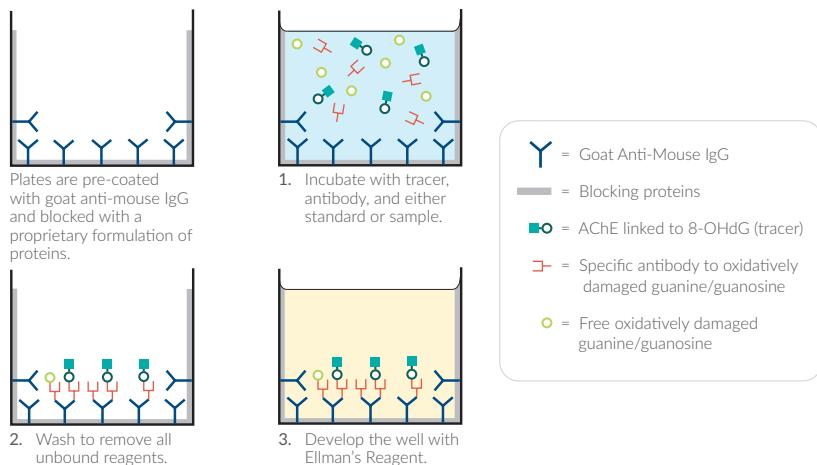


Figure 2. Schematic of the AChE ELISA

Definition of Key Terms

Blk (Blank): background absorbance caused by Ellman's Reagent. The blank absorbance should be subtracted from the absorbance readings of all the other wells, including the non-specific binding (NSB) wells.

TA (Total Activity): total enzymatic activity of the AChE-linked tracer.

NSB (Non-Specific Binding): non-immunological binding of the tracer to the well. Even in the absence of specific antibody a very small amount of tracer still binds to the well; the NSB is a measure of this low binding.

B₀ (Maximum Binding): maximum amount of the tracer that the antibody can bind in the absence of free analyte.

%B/B₀ (%Bound/Maximum Bound): ratio of the absorbance of a particular sample or standard well to the average absorbance of the maximum binding (B₀) wells.

Standard Curve: a plot of the %B/B₀ values *versus* concentration of a series of wells containing known amounts of analyte.

Dtn (Determination): one dtn is the amount of reagent used per well.

Cross Reactivity: numerical representation of the relative reactivity of this assay towards structurally related molecules as compared to the primary analyte of interest. Biomolecules that possess similar epitopes to the analyte can compete with the tracer for binding to the primary antibody. Substances that are superior to the analyte in displacing the tracer result in a cross reactivity that is greater than 100%. Substances that are inferior to the primary analyte in displacing the tracer result in a cross reactivity that is less than 100%. Cross reactivity is calculated by comparing the mid-point (50% B/B₀) value of the tested molecule to the mid-point (50% B/B₀) value of the primary analyte when each is measured in assay buffer using the following formula:

$$\% \text{ Cross Reactivity} = \left[\frac{50\% \text{ B/B}_0 \text{ value for the primary analyte}}{50\% \text{ B/B}_0 \text{ value for the potential cross reactant}} \right] \times 100\%$$

LLOD (Lower Limit of Detection): the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as a concentration two standard deviations higher than the mean zero value.

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for at least two months. NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with ultrapure water.

1. Assay Buffer Preparation

Dilute the contents of one vial of ELISA Buffer Concentrate (10X) (Item No. 400060) with 90 ml of ultrapure water. Be certain to rinse the vial to remove any salts that may have precipitated.

2. Wash Buffer (1X) Preparation

Dilute the contents of one vial of Wash Buffer Concentrate (400X) (Item No. 400062) with ultrapure water to a total volume of 2 L and add 1 ml of Polysorbate 20 (Item No. 400035). NOTE: Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.

Sample Preparation

This assay has been validated in plasma, serum, urine, saliva, and cell culture medium. Other sample types should be tested for interference to evaluate the need for sample purification before embarking on a large number of sample measurements.

Testing for Interference

To test for interference, assay one or two samples at a range of dilutions, then select at least two different dilutions of each sample within the linear portion of the standard curve. If two different dilutions of the same sample show good correlation (differ by 20% or less) in the final calculated oxidized guanine/guanosine concentration, sample purification is not required. If you do not see good correlation of the different dilutions, purification is advised. Purification methods will need to be determined by the user.

General Precautions

- All samples must be free of organic solvents prior to assay.
- Samples that cannot be assayed immediately should be stored as indicated in the sample preparation.
- Samples of mouse or rat origin may contain antibodies which interfere with the assay by binding to the anti-mouse plate. We recommend that all mouse and rat samples be purified prior to use in this assay.

Urine

Urine samples should be assayed immediately after collection or stored at -80°C. Interference in urine is infrequent. Dilute urine samples with Assay Buffer at least 1:50 to fall within the range of the standard curve. Urinary concentrations of oxidized guanine/guanosine species vary considerably. It is recommended that the values obtained from urine samples be standardized to creatinine levels using Cayman's Creatinine ELISA Kit (Item No. 502330), Creatinine (urinary) Colorimetric Assay Kit (Item No. 500701), or a similar assay.

Saliva

Saliva samples should be assayed immediately after collection or stored at -80°C. Dilute samples with Assay Buffer prior to the assay.

Plasma/Serum

Plasma and serum samples should be assayed immediately after collection or stored at -80°C. It is recommended to dilute plasma or serum at least 1:10 with Assay Buffer to fall within the range of the assay.

Culture Medium Samples

Collect culture medium samples and assay immediately after collection or stored at -80°C. Fetal bovine serum contains oxidized guanine/guanosine species, therefore assays should be performed in serum-free medium or PBS.

Serum-free medium interferes with the assay at a concentration of 20% and above. To eliminate this interference, the samples should be diluted at a minimum of 1:5. If samples need to be tested at the lower dilution, prepare a standard curve in the Assay Buffer containing the same concentration of cell culture medium as in samples to be tested. (*i.e.*, if samples are diluted 1:2 in assay buffer, the standard curve must be prepared in 50% cell culture medium and 50% assay buffer.)

DNA Preparation

The following protocols are suggested guidelines to purify and digest DNA for use in this kit. These protocols have not been validated. The amount of DNA needed to detect 8-OHdG will vary depending upon the degree of oxidative damage.

Cell Lysates

Lyse cells using established methods and store at -80°C until use. 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

Snap-freeze tissue samples in liquid nitrogen immediately after collection. Store at -80°C until use. 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. Process as described for cell lysates.

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

DNA/RNA Oxidative Damage Express ELISA Standard

Reconstitute one vial of the DNA/RNA Oxidative Damage Express ELISA Standard (Item No. 401157) with 1 ml of Assay Buffer. The concentration of this solution (the bulk standard) will be 100 ng/ml. It will be stable for approximately 1 week when stored at 4°C.

NOTE: If assaying culture medium samples that have not been diluted at least 1:5 with Assay Buffer, culture medium solution in Assay Buffer should be used for dilution of the standard curve (see Culture Medium Samples preparation on page 14).

To prepare the standard for use in ELISA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 900 µl Assay Buffer to tube #1 and 500 µl Assay Buffer to tubes #2-8. Transfer 100 µl of the bulk standard (100 ng/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 400 µl from tube #1 and placing in tube #2; mix thoroughly. Next, remove 400 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8.

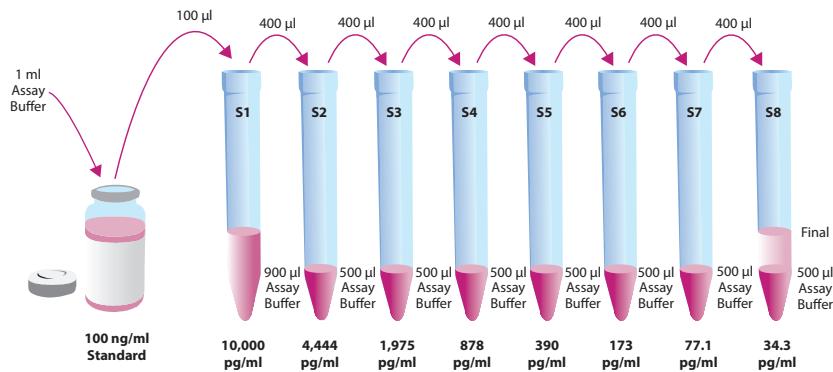


Figure 3. Preparation of the DNA/RNA Oxidative Damage Express standards

DNA/RNA Oxidative Damage Express ELISA AChE Tracer

Reconstitute the DNA/RNA Oxidative Damage Express AChE Tracer (Item No. 401156) with 6 ml of Assay Buffer. Store the reconstituted DNA/RNA Oxidative Damage Express ELISA AChE Tracer at 4°C (*do not freeze!*) and use within two weeks. A 20% surplus of tracer has been included to account for any incidental losses.

Tracer Dye Instructions (optional)

This dye may be added to the tracer, if desired, to aid in visualization of tracer-containing wells. Add the dye to the reconstituted tracer at a final dilution of 1:100 (add 10 µl of dye per 1 ml tracer). Do not store with dye.

DNA/RNA Oxidative Damage Express ELISA Antibody

Reconstitute the DNA/RNA Oxidative Damage Express ELISA Monoclonal Antibody (Item No. 401155) with 6 ml of Assay Buffer. Store the reconstituted DNA/RNA Oxidative Damage Express ELISA Antibody at 4°C. It will be stable for two weeks. A 20% surplus of antibody has been included to account for any incidental losses.

Antiserum Dye Instructions (optional)

This dye may be added to the antibody, if desired, to aid in visualization of antiserum-containing wells. Add the dye to the reconstituted antibody at a final dilution of 1:100 (add 10 µl of dye per 1 ml tracer). Do not store with dye.

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 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain a minimum of two Blk, two NSB, and three B₀, and an eight-point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* Each sample should be assayed at a minimum of two dilutions and each dilution should be assayed at least in duplicate. For statistical purposes, assaying samples in triplicate is recommended.

A suggested plate format is shown in Figure 4, 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 using a convenient spreadsheet offered by Cayman (see page 22, for more details). It is suggested that the contents of each well be recorded on the template sheet provided (see page 29).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blk	S1	S1	1	1	1	9	9	9	17	17	17
B	Blk	S2	S2	2	2	2	10	10	10	18	18	18
C	NSB	S3	S3	3	3	3	11	11	11	19	19	19
D	NSB	S4	S4	4	4	4	12	12	12	20	20	20
E	B ₀	S5	S5	5	5	5	13	13	13	21	21	21
F	B ₀	S6	S6	6	6	6	14	14	14	22	22	22
G	B ₀	S7	S7	7	7	7	15	15	15	23	23	23
H	TA	S8	S8	8	8	8	16	16	16	24	24	24

Blk = Blank Wells
TA = Total Activity Wells
NSB = Non-Specific Binding Wells
B₀ = Maximum Binding Wells
S1-S8 = Standard Wells
1-24 = Sample Wells

Figure 4. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Addition of the Reagents

1. Assay Buffer

Add 100 µl Assay Buffer to NSB wells. Add 50 µl Assay Buffer to B₀ wells. If culture medium solution in Assay Buffer was used to dilute the standard curve (see page 14 for details), add 50 µl of the same solution to NSB and B₀ wells in place of Assay Buffer.

2. DNA/RNA Oxidative Damage Express ELISA Standard

Add 50 µl from tube #8 to both of the lowest standard wells (S8). Add 50 µl from tube #7 to each of the next two standard wells (S7). Continue with this procedure until all the standards are aliquoted. The same pipette tip should be used to aliquot all the standards. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard.

3. Samples

Add 50 µl of sample per well.

4. DNA/RNA Oxidative Damage Express ELISA AChE Tracer

Add 50 µl to each well *except* the TA and the Blk wells.

5. DNA/RNA Oxidative Damage Express ELISA Antibody

Add 50 µl to each well *except* the TA, NSB, and the Blk wells within 15 minutes of adding the tracer.

Well	ELISA Buffer	Standard/ Sample	Tracer	Antibody
Blk	-	-	-	-
TA	-	-	5 μ l (at devl. step)	-
NSB	100 μ l	-	50 μ l	-
B ₀	50 μ l	-	50 μ l	50 μ l
Standard/ Sample	-	50 μ l	50 μ l	50 μ l

Table 1. Pipetting summary

Incubation of the Plate

Cover each plate with a 96-Well Cover Sheet (Item No. 400012) and incubate two hours at room temperature on an orbital shaker.

Development of the Plate

1. Reconstitute 100 dtn vial of Ellman's Reagent (Item No. 400050) with 20 ml of ultrapure water immediately before use.

NOTE: Reconstituted Ellman's Reagent is unstable and should be used the same day it is prepared; protect the Ellman's Reagent from light when not in use. Extra vials of the reagent have been provided should a plate need to be re-developed or multiple assays run on different days.

2. Empty the wells and rinse five times with ~300 μ l of Wash Buffer (1X).
3. Add 200 μ l of Ellman's Reagent to each well.
4. Add 5 μ l of the reconstituted tracer to the TA wells.
5. Cover the plate with the 96-Well Cover Sheet. Optimum development is obtained by using an orbital shaker equipped with a large, flat cover to allow the plate(s) to develop in the dark. This assay typically develops (*i.e.*, B₀ wells \geq 0.3 A.U. (blk subtracted)) in 60-90 minutes.

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Remove the plate cover being careful to keep Ellman's Reagent from splashing on the cover. *NOTE: Any loss of Ellman's Reagent will affect the absorbance readings.*
3. Read the plate at a wavelength between 405 and 420 nm. The absorbance may be checked periodically until the B₀ wells have reached a minimum of 0.3 A.U. (Blk subtracted). The plate should be read when the absorbance of the B₀ wells are in the range of 0.3-2.0 A.U. (Blk subtracted). If the absorbance of the wells exceeds 2.0, wash the plate, add fresh Ellman's Reagent and let it develop again.

ANALYSIS

Many plate readers come with data reduction software that plot data automatically. Alternatively a spreadsheet program can be used. The data should be plotted as either %B/B₀ versus log concentration using a four-parameter logistic fit or as logit B/B₀ versus log concentration using a linear fit. *NOTE: Cayman has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/elisa) to obtain a free copy of this convenient data analysis tool.*

Calculations

Preparation of the Data

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

NOTE: If the plate reader has not subtracted the absorbance readings of the blank wells from the absorbance readings of the rest of the plate, be sure to do that now.

1. Average the absorbance readings from the NSB wells.
2. Average the absorbance readings from the B₀ wells.
3. Subtract the NSB average from the B₀ average.
4. Calculate the B/B₀ (Sample or Standard Bound/Maximum Bound) for the remaining wells. To do this, subtract the average NSB absorbance from the S1 absorbance and divide by the corrected B₀ (from Step 3). Repeat for S2-S8 and all sample wells. (To obtain %B/B₀ for a logistic four-parameter fit, multiply these values by 100.)

NOTE: The TA values are not used in the standard curve calculations. Rather, they are used as a diagnostic tool. Low or no absorbance from a TA well could indicate a dysfunction in the enzyme-substrate system.

Plot the Standard Curve

Plot %B/B₀ for standards S1-S8 versus 8-hydroxy-2'-deoxyguanosine concentration using linear (y) and log (x) axes and perform a 4-parameter logistic fit.

Alternative Plot - The data can also be linearized using a logit transformation. The equation for this conversion is shown below. *NOTE: Do not use %B/B₀ in this calculation.*

$$\text{logit (B/B}_0\text{)} = \ln [\text{B/B}_0\text{}/(1 - \text{B/B}_0\text{)}]$$

Plot the data as logit (B/B₀) versus log concentrations and perform a linear regression fit.

Determine the Sample Concentration

Calculate the %B/B₀ (or B/B₀) value for each sample. Determine the concentration of each sample using the equation obtained from the standard curve plot. *NOTE: Remember to account for any concentration or dilution of the sample prior to the addition to the well. Samples outside of the linear range of standard curve need to be re-assayed. A 20% or greater disparity between the apparent concentration of two different dilutions of the same sample indicates interference which could be eliminated by purification.*

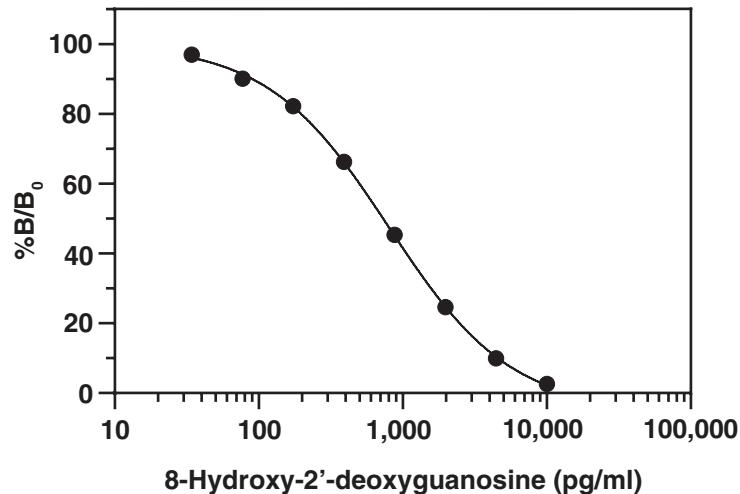
Performance Characteristics

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data below to determine the values of your samples.

DNA/RNA Oxidative Standard (pg/ml)	Blk-subtracted Absorbance	NSB-corrected Absorbance	%B/B ₀	%CV* Intra-assay Precision	%CV* Inter-assay Precision
NSB	0.012	--	--	--	--
B ₀	0.965	0.953	--	--	--
TA	0.632	--	--	--	--
10,000	0.037	0.025	2.6	8.5	8.6
4,444	0.107	0.095	10.0	8.9	5.4
1,975	0.246	0.234	24.6	4.3	1.7
878	0.444	0.432	45.3	5.9	2.6
390	0.643	0.631	66.2	9.2	1.6
173	0.795	0.783	82.2	20.0	4.8
77.1	0.870	0.858	90.0	44.7**	12.6
34.3	0.936	0.924	97.0	54.8**	17.0

Table 2. Typical results

*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve. **Evaluate data in this range with caution.



Assay Range = 34.3-10,000 pg/ml
Sensitivity (defined as 80% B/B₀) = 175 pg/ml
Mid-point (defined as 50% B/B₀) = 738 pg/ml
Lower Limit of Detection (LLOD) = 62 pg/ml

The sensitivity and mid-point were derived from the standard curve shown above. The standard was diluted with Assay Buffer.

Figure 5. Typical standard curve

Cross Reactivity:

Compound	Cross Reactivity
8-hydroxy-2'-deoxyguanosine	100%
8-hydroxyguanosine	31%
8-hydroxyguanine	29%
Guanosine	<0.01%

Table 3. Cross Reactivity of the DNA/RNA Oxidative Damage Express ELISA

RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of replicates	A. Trace organic contaminants in the water B. Poor pipetting/technique
High NSB (>10% of B ₀)	A. Poor washing B. Exposure of NSB wells to specific antibody
Poor development (low signal) of standard curve	A. Trace organic contaminants in the water B. Dilution error in preparing reagents
Poor development (low signal) of samples	A. AChE inhibitors are present; ensure that the samples and buffers are free of AChE inhibitors B. Sample requires further dilution
Analyses of two dilutions of a biological sample do not agree (<i>i.e.</i> , more than 20% difference)	Interfering substances are present; determine minimal dilution for that sample type
Only Total Activity (TA) wells develop.	Trace organic contaminants in the water OR omission of antibody or tracer

References

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2. Shi, F., Nie, B., Gan, W., *et al.* Oxidative damage of DNA, RNA and their metabolites in leukocytes, plasma and urine of *Macaca mulatta*: 8-oxoguanosine in urine is a useful marker for aging. *Free Radic. Res.* **46(9)**, 1093-1098 (2012).
3. Roszkowski, K. and Olinski, R. Urinary 8-oxoguanine as a predictor of survival in patients undergoing radiotherapy. *Cancer Epidemiol. Biomarkers Prev.* **21**, 629-634 (2012).
4. Santos, R.X., Correia, S.C., Zhu, X., *et al.* Nuclear and mitochondrial DNA oxidation in Alzheimer's disease. *Free Radical Research* **46(4)**, 565-576 (2012).
5. Li, Y. and Wang, X. The role of DNA and RNA guanosine oxidation in cardiovascular disease. *Pharm. Res.* **107187** (2024).

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

Warranty and Limitation of Remedy

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