LSBio

an Absolute Biotech Company

Oxidative DNA Damage / 8-OH-dG ELISA Kit (Competitive EIA)

User Manual

Catalog No. LS-F20632

It is important that you read this entire manual carefully before starting your experiment.

This kit is for Research Use Only. Not for Diagnostic Use.

This kit is not approved for use in humans or for clinical diagnosis.

Assay Specifications

Target:	Oxidative DNA Damage / 8-OH-dG
Specificity:	8-hydroxy-2-deoxy Guanosine ... 100%
	8-hydroxy Guanosine 23%
	8-hydroxy Guanine 23%
	Guanosine ..... <0.01%
Sample Types:	This kit is recommended for use with samples such as urine, cell culture, and plasma.
Detection 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:	Inter-Assay: <10%; Intra-Assay: <10%
	See **Performance Characteristics** for more details
Limitations:	This kit is for **Research Use Only** and is not intended for diagnostic use. This kit is not approved for use in humans or for clinical diagnosis.

Kit Components

Component	Quantity
8-OH-dG BSA Coated Strip Plate	1
8-OH-dG Standard	1 vial/100µl
8-OH-dG HRP Conjugated Monoclonal Antibody	1 vial/75µl
Sample Diluent (Red)	1 vial/50mL
8-OH-dG Antibody Diluent (Blue)	1 vial/13mL
Wash Buffer Concentrate (10X)	1 vial/ 50mL
TMB Substrate	1 vial/13mL
Stop Solution	1 vial/13ml
Adhesive Plate Sealer	4
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Kit Storage

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 (10 µL of Standard can prepare a triplicate standard curve.

Other Required Supplies

- Microplate reader with 450nm wavelength filter
- High-precision pipette and sterile pipette tips
- Deionized or distilled water
- Additional materials see the **Sample Preparation** section.

Background

8-hydroxy-2-deoxy Guanosine (8-OHdG) is produced by the oxidative damage of DNA (see Figure 1, on page 4) 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

LSBio'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.

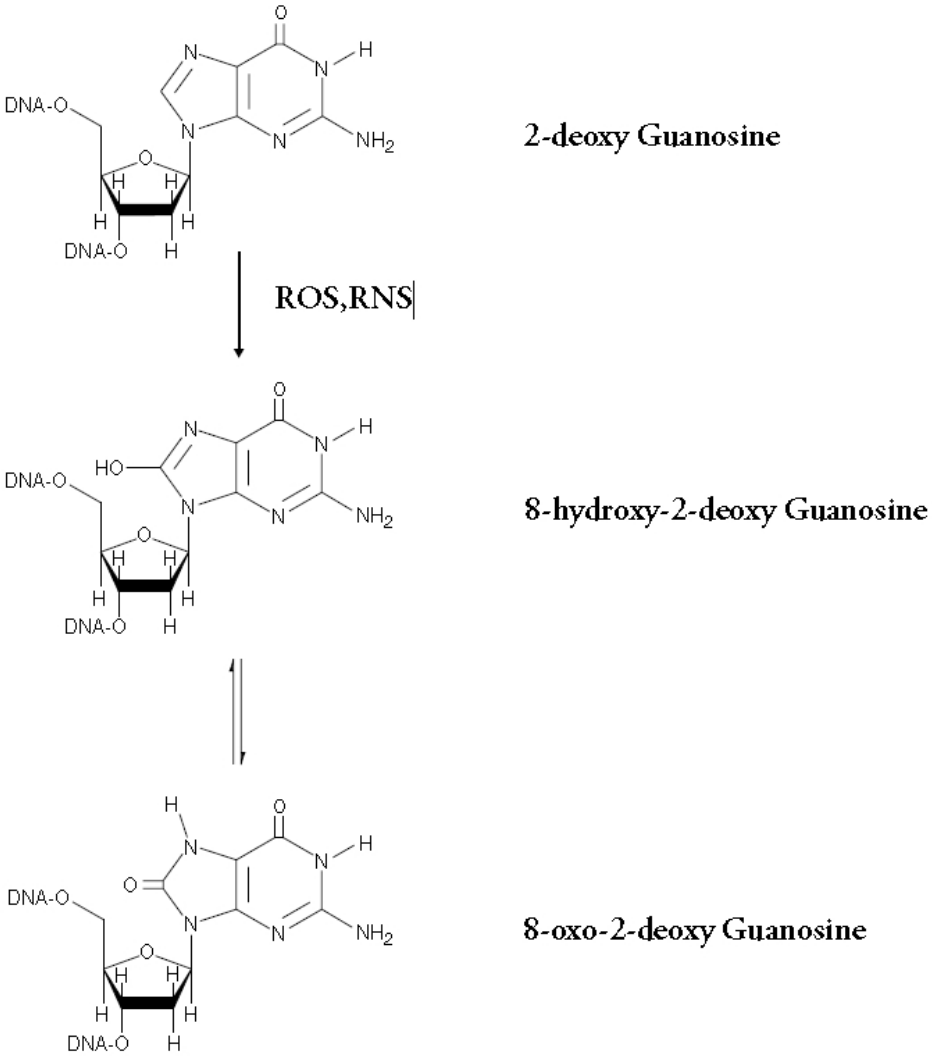
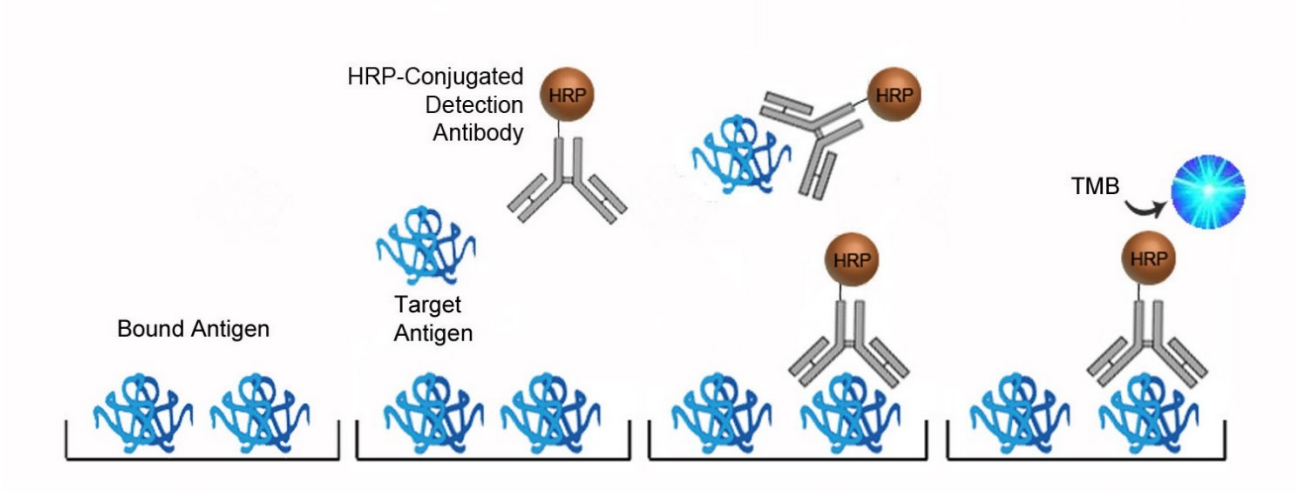


Figure 1: Oxidation of Guanosine

Assay Principle

This assay is based on the competition ELISA principle. Each well of the supplied microtiter plate has been pre-coated with the target antigen. Standards or samples are added to the wells as well as a fixed quantity of Horseradish Peroxidase (HRP)-conjugated detection antibody. The free antigens (in the standards or samples) and antigens bound to the plate compete to bind to the detection antibody. All but the bound HRP-conjugated detection antibody is washed away. A TMB substrate is then added which reacts with the HRP enzyme resulting in color development. A sulfuric acid stop solution is added to terminate color development reaction and then the optical density (OD) of the well is measured at a wavelength of 450 nm ± 2 nm. The OD of an unknown sample can then be compared to an OD standard curve generated using known antigen concentrations in order to determine its antigen concentration. In contract to typical Sandwich ELISA assays, in competition assay the greater the amount of antigen in the sample, the lower the color development and optical density reading.

A schematic of this process is shown on the next page.


HRP-Conjugated
Detection
Antibody
HRP
HRP
TMB
HRP
HRP
Target
Antigen
Bound Antigen

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 180 µL of your diluted sample to permit assay in triplicate (approximately 50 µL/ 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-OHdG can vary considerably and can be standardized against creatinine levels if required.

Storage: Fresh urine samples should be centrifuged at 2,000 x g for 10 minutes or filtered with a 0.2 µm filter before this assay, and stored at -20°C immediately after collection.

Dilution: Dilute urine samples 1:20 (v:v) in Sample and Standard Diluent as the 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 DNA-incorporated 8-OHdG. Glomerular filtration results in excretion of 8-OHdG into the urine, while the DNA-incorporated 8-OHdG remains in the blood. The differing 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.

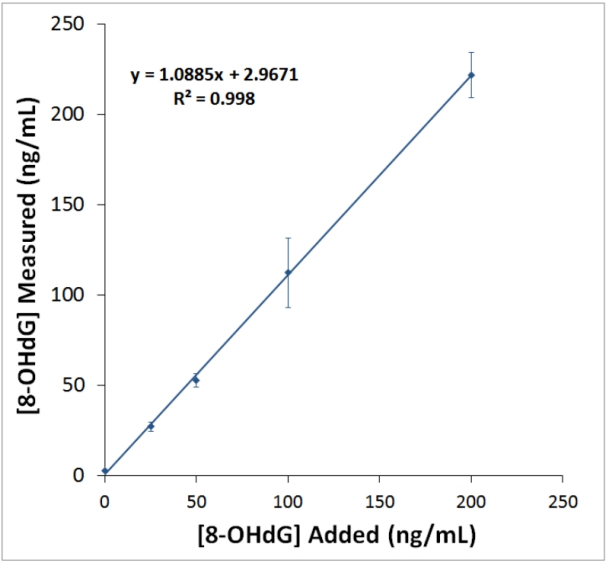


Figure 3. Recovery of 8-hydroxy-2-deoxy Guanosine from urine

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.

Standard Preparation

NOTE: The Standard should be aliquotted 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 and 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. Similarly, 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).

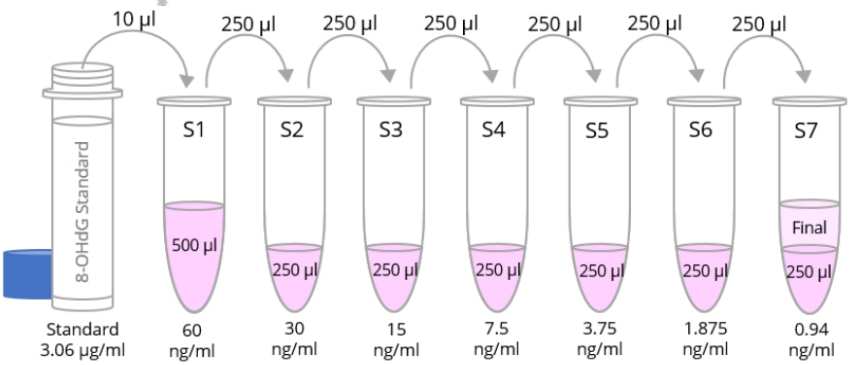


Figure 4: Preparation of the 8-hydroxy-2-deoxy guanosine standards

Reagent Preparation

1X Wash Buffer Preparation

Prepare 1X Wash buffer by diluting 10X Wash Buffer in distilled or deionized water. For example, if preparing 500 mL 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-hydroxy2-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.

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

S1 – S7: 60 to 0.94 ng/ml Standards **Blk:** Blank
S8: Zero Standard **1 – 23:** Samples

Figure 5: Sample plate format

Assay Procedure

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 7-9) 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

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

Develop the Plate

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

Many plate readers come with data reduction software that plot data automatically. Alternatively a spreadsheet program can be used.

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

Sample Data

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 this one to determine the values of your samples. Depending on the development conditions and the purity of the water used, your results could differ substantially from the data presented below.

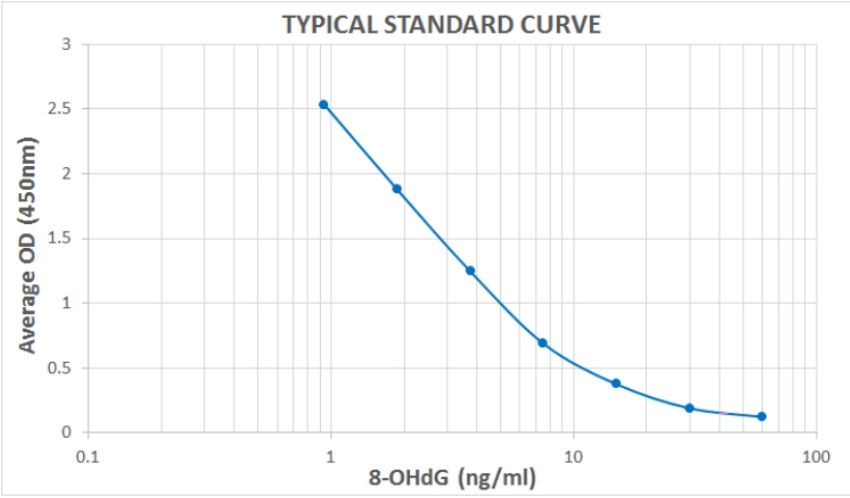
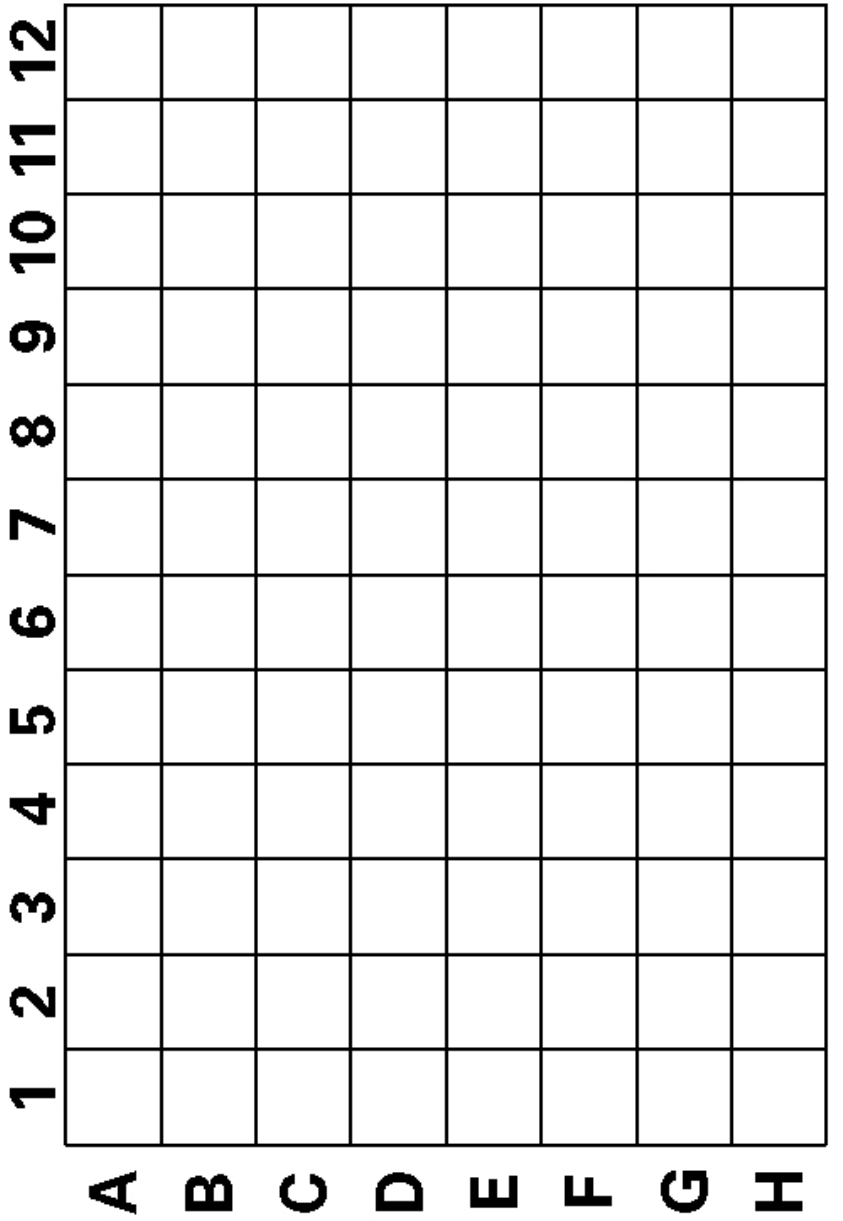


Figure: Typical standard curve

Troubleshooting Guide

Problem	Possible Cause	Recommended Solutions
Poor Standard Curve	1. Improper standard solution 2. Standard degraded 3. Curve doesn't fit scale 4. Pipetting Error	1. Confirm dilutions are made correctly. 2. Store and handle standard as recommended. 3. Try plotting using different scales 4. Use calibrated pipettes and proper pipetting technique.
No Signal	1. Plate washings too vigorous 2. Wells dried out 3. Target present below detection levels of kit	1. Check and ensure correct pressure in automatic wash system. Pipette wash buffer gently if washes are done manually. 2. Do not allow wells to dry out. Cover the plate for incubations. 3. The basic range of DNA to use, if the damage is low, 100 ug/ml - if maximally damaged,1 ng/ml and dilute from there.
High Background	1. Wells are insufficiently washed 2. Contaminated wash buffer 3. Waiting too long to read the plate after adding stop solution	1. Wash wells as per protocol 2. Prepare fresh wash buffer 3. Read plate immediately
Low Sensitivity	1. Standard is degraded 2. Mixing or substituting reagents from other kits	1. Replace standard 2. Avoid mixing components

Template Sheet



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ASSAY USAGE AND SUPPORT

This kit is for **Research Use Only** and is not intended for diagnostic use. This kit is not approved for use in humans or for clinical diagnosis. This kit should not be used beyond the expiration date printed on the lot specific kit label.

Warning: This reagent may contain sodium azide and sulfuric acid. The chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation, and ingestion. Sodium azide forms hydrazoic acid under acidic conditions and may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent accumulation.

The LifeSpan Guarantee: LifeSpan guarantees the integrity of all components contained with an immunoassay kit, and that the standards provided will produce a standard curve sufficient for the quantification of target antigen concentrations that fall within the specified range of the kit. Due to the variable nature of sample types and preparations, LifeSpan cannot guarantee that the target antigen will be detectable in customer-supplied samples. For this reason, LifeSpan strongly recommends that customers conduct validation experiments, using positive control samples generated in a similar manner to the experimental samples, before using valuable research specimens. Due to the perishable nature of ELISA kits, orders of greater than 5 units of a single catalog number cannot be returned upon shipment, and are not eligible for refund.

Technical Support: LifeSpan's knowledgeable staff scientists are available to answer any questions about this kit. Email your detailed questions to Technical.Support@LSBio.com.

RETURNS, REFUNDS, CANCELLATIONS

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