# 8-Hydroxy-2’-deoxyguanosine (8-OHdG)

# DNA Damage ELISA kit

# (96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-55100-96

www.zellx.de

Sample Types Validated for:

Serum, EDTA and Heparin Plasma, Saliva, Urine, Digested DNA, Fecal Extracts and Tissue Culture Media

**!!! Caution: This product is for Research Use Only. Not for *in vitro* Diagnostics !!!**

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Please read this insert completely prior to using the product.


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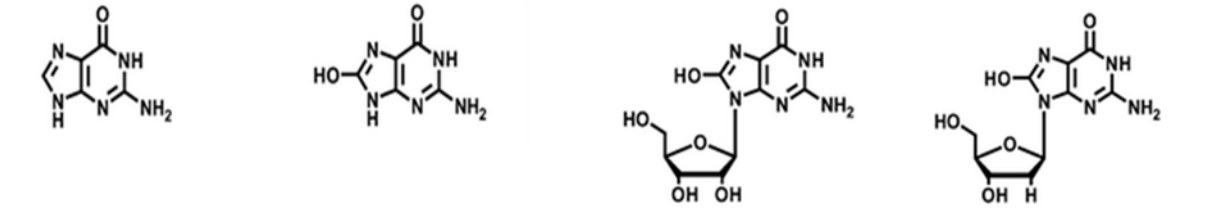
# Introduction

## Background

Free radicals and reactive oxygen species (ROS) are constantly generated *in vivo* during the normal metabolism, or due to ultraviolet and ionizing radiation. These endogenously- and exogenously- produced ROS can attack lipids, proteins and nucleic acids in living cells, and cause damage.

The process of oxidative damage in DNA results in the formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG), which is a regarded as a ubiquitous marker of oxidative stress. When individual bases are damaged, nonspecific DNA repair enzymes and base specific repair glycosylases excise DNA lesions and release deoxynucleotides, which are enzymatically hydrolyzed to stable deoxynucleosides. These repair products are transported through the blood and excreted in the urine. 8-OHdG is an oxidized derivative of deoxyguanosine that is formed during the repair of damaged DNA by exonucleases, and further excreted into urine. Damage to RNA is reflected in nucleoside adducts such as 8-Hydroxyguanosine (8-OHG).

It is widely thought that continuous oxidative damage to DNA is a significant contributor to age-related development of the major cancers, such as those of the colon, breast, rectum, and prostate. 8-OHdG is physiologically formed and enhanced by chemical carcinogens.



Guanine | Hydroxyguanine | 8-Hydroxyguanosine (8-OHG) | 8-hydroxy-2'-deoxyguanosine (8-OHdG)

## Assay principle

The ZellX® 8-OHdG ELISA Kit is designed to quantitatively measure oxidized guanosine species derived from damaged DNA and RNA molecules. The assay detects all three oxidized derivatives including 8-hydroxy-2'-deoxyguanosine (8-OHdG), 8-hydroxyguanosine (8-OHG), and 8-hydroxyguanine which may be present in serum, plasma, saliva, urine, dried fecal samples, and tissue culture media samples.

The kit includes a 96-well plate that is pre-coated with a secondary rabbit anti-mouse antibody. The function of this antibody is to capture the mouse anti-8-OHdG antibody bound to 8-OHdG conjugate (peroxidase-labeled) and hold this complex to the plate during the subsequent detection steps. The 8-OHdG-conjugate (labeled) and the sample 8-OHdG (unlabeled) compete for binding to the mouse

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antibody. After 2 hours of incubation, the substrate is added to react with the peroxidase-labeled antibody-antigen conjugate. After stopping the reaction, the intensity of the generated color can be measured at 450 nm. The lower the amount of 8-OHdG in the sample, the stronger the signal due to more labeled 8-OHdG bound to the well. An 8-OHdG stock solution is provided to generate a standard curve for the assay and all samples should be read off the standard curve.

# General information

## Materials supplied in the Kit

| *Component* | *Quantity* |
|---|---|
| **8-OHdG Standard (160 ng/mL)** | 70 µL |
| **8-OHdG Antibody** | 2.6 mL |
| **8-OHG Conjugate** | 2.6 mL |
| **Assay Buffer Concentrate (5x)** | 11 mL |
| **Wash Buffer Concentrate (20x)** | 25 mL |
| **TMB Substrate** | 11 mL |
| **Stop Solution** | 5 mL |
| **Coated Clear Half Area 96 Well Plate & Sealer** | 1 plate |

## Storage instruction

All reagents should be stored at 4° C until the expiration date of the kit.

## Materials required but not supplied

Deionized water (di$H_2O$)

Phosphate Buffer Saline (PBS)

Microplate/ELISA reader capable of reading optical absorption at 450 nm

Centrifuge, Vortex mixer

Precision pipettes, multichannel/repeater pipettes and disposable pipette tips

Disposable 1.5-2 mL microtubes for sample preparation

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**For Dried Fecal Sample:**

ACS Grade Ethanol

Glass test tubes

**For DNA Sample:**

Nuclease P1 (Sigma-Aldrich N8630)

Sodium Acetate (3 M pH 5.2)

Tris (1 M pH 7.5)

Alkaline Phosphatase (NEB M02905), 10 U/mL aliquots in -20˚C

Zinc Chloride (Sigma-Aldrich 39059)

## Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

Stop Solution is an acidic solution and should not come in contact with skin or eyes. Handling this reagent needs appropriate precaution.

## General remarks

- Equilibrate all kit components to room temperature (RT) 30 minutes before use.
- The instruction must be strictly followed.
- The reading of Microplate/ELISA reader must be set at the appropriate wavelength.
- Pipette tips should not be used more than once in order to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.
- The antibody coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.
- This kit utilizes a peroxidase-based readout system. Buffers, including Wash Buffers from other manufacturers, containing sodium azide will inhibit color production from the enzyme. Make sure all buffers used for samples are azide free. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer.

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# Assay protocol

## Reagent preparation

i. **Assay Buffer:** Prepare a 1:5 dilution of Assay Buffer Concentrate with di$H_2O$ (1 part Assay Buffer Conc. with 4 parts d$IH_2O$), and mix well. Assay Buffer can be stored at 4°C for up to 3 months.

ii. **Wash Buffer:** Prepare a 1:20 dilution of Wash Buffer Concentrate with di$H_2O$ (1 part Wash Buffer Conc. with 19 parts d$IH_2O$), and mix well. Assay Buffer can be stored at room temperature for up to 3 months.

## Sample preparation

Since 8-Hydroxy-2'-deoxyguanosine (8-OHdG) is identical across all species, it is expected that this kit can measure 8-OHdG in all other species.

Samples containing visible particulate should be centrifuged prior to conducting the assay. Moderate to severely hemolyzed samples should not be used for this assay.

**All samples and standards must be used within 2 hours of preparation.**

I. **Serum, Plasma:**

- Collect the fresh serum or plasma with heparin or EDTA.
- Centrifuge at 600 g for 10 min at 4˚C.
- Transfer the serum or plasma from the red blood cells into fresh tubes.
- Samples that are not clear or contain visible particulate should be centrifuged prior to using.
- Serum should be diluted ≥ 1:8 by taking one part of serum and adding 7 or more parts of Assay Buffer prior to conducting assay.
- Experiment should be conducted immediately. Otherwise, aliquots of the sample should be kept at -70˚C or lower temperature.

II. **Urine:**

- Urine should be diluted ≥ 1:4 by taking one part of sample and adding 3 or more parts of Assay Buffer prior to conducting assay.
- **Normalize the sample value based on creatinine levels using our Urine Creatinine assay kit Cat NO. ZX-44110-96 in random urine specimen.**

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III. **Saliva:**

- Saliva sample should be diluted ≥ 1:2 by taking one part of sample and adding 1 or more parts of Assay Buffer prior to conducting assay.

IV. **Dried Fecal Sample:**

- Ensure that the sample is completely dry, and powder the sample to improve extraction recovery. Remove any large particles if possible.
- Weigh out ≥ 0.2 gram of dried fecal solid into a tube. Samples can be dried by passive drying, gentle heating (≤ 60°C), or freeze-drying (lyophilization).
- Add 1 mL of ethanol per 0.1 gram of solid fecal sample (100 mg/mL) and seal.
- Shake strongly for at least 30 minutes.
- Centrifuge at 5000 rpm at 4°C for 15 minutes and collect supernatant in a clean tube. This material can be stored at ≤ -20°C for at least a month if properly sealed.
  - **Note:** Samples containing low levels of analyte can be concentrated by drying down the extract and resuspension in a reduced volume of Assay Buffer.
- Supernatant should be diluted ≥ 1:5 by taking one part of sample and adding 4 or more parts of Assay Buffer.
- Vortex well and allow to rest 5 minutes at room temperature
- Repeat the vortex step 2 more times to ensure complete steroid solubility.
- The final concentration of ethanol in the sample to be added to the wells should be ≤ 2.5%. **(≥ 1:8 dilution with Assay Buffer is needed.)**

V. **DNA sample:**

- Purify DNA from cell or tissue samples by a desired method or commercial DNA purification kit.
- Prepare the DNA sample by adding 15 µg of DNA in 100 µL di$H_2O$. (These volumes can be scaled up).
- Prepare a working solution of Nuclease P1 at 5 U/mL in 40 mM sodium acetate and keep on ice.
- Remove an aliquot of alkaline phosphatase, 10 U/mL, from -20˚C. Keep on ice.
- Denature the DNA at 95-100˚C for 10 min. Cool completely on ice for 5 min. Centrifuge for 5 sec or tap any condensate down into tube.
- Add 50 µL of 40 mM sodium acetate with 0.4 mM Zinc Chloride (pH 5.0-5.4).
- Add 50 µL of 5 U/mL Nuclease P1. Invert the tube to mix. Centrifuge 5 seconds or tap any condensate down into tube.
- Incubate at 37˚C for 30 min.
- Adjust pH to 7.5-8.0 by adding 20 µL of 1M Tris pH 7.5 to tube.
- Add 15 µL of 10 U/mL alkaline phosphatase. Invert to mix. Centrifuge 5 sec or tap any condensate down into tube.
- Incubate at 37˚C for 30 min.

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- Boil samples for 10 min at 95˚C to inactivate alkaline phosphatase. Place samples on ice.
- Samples should be diluted ≥ 1:4 with the Assay Buffer prior to conducting the assay.

VI. **Tissue Culture Media:**

- Tissue culture media should be prepared with the diluted Assay Buffer prior to conducting assay.

**All the samples must be used within 2 hours of preparation; otherwise, aliquots of the sample should be kept at ≤ -20˚C for later use.**

## Standard preparation

- Prepare a 1:20 dilution of 8-OHdG Standard with Assay Buffer (mix 25 µL of standard with 475 µL of Assay Buffer), and label as the Standard No.8 (8000 pg/mL).
- Make series of lower dilutions as described in the table.
- The Assay Buffer is used as the 0 pg/mL standard.

| *No.* | *Concentration* | *Material needed* |
|---|---|---|
| **Standard No.8** | 8000 pg/mL | 25 µL 8-OHdG Standard + 475 µL Assay Buffer |
| **Standard No.7** | 4000 pg/mL | 250 µL Standard No.8 + 250 µL Assay Buffer |
| **Standard No.6** | 2000 pg/mL | 250 µL Standard No.7 + 250 µL Assay Buffer |
| **Standard No.5** | 1000 pg/mL | 250 µL Standard No.6 + 250 µL Assay Buffer |
| **Standard No.4** | 500 pg/mL | 250 µL Standard No.5 + 250 µL Assay Buffer |
| **Standard No.3** | 250 pg/mL | 250 µL Standard No.4 + 250 µL Assay Buffer |
| **Standard No.2** | 125 pg/mL | 250 µL Standard No.3 + 250 µL Assay Buffer |
| **Standard No.1** | 62.5 pg/mL | 250 µL Standard No.2 + 250 µL Assay Buffer |
| **Standard No.0** | 0 pg/mL | 250 µL Assay Buffer |

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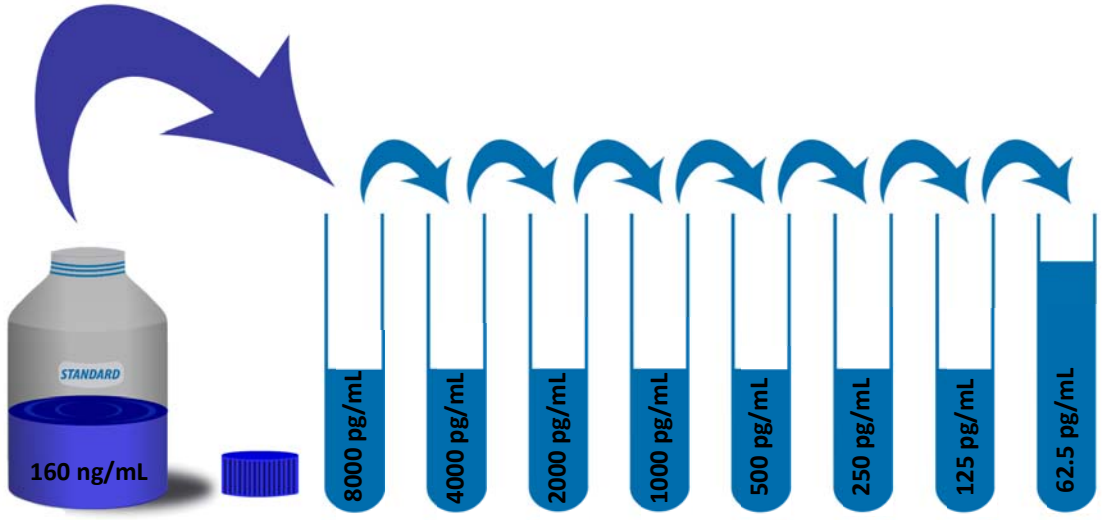


**All standard must be used within 2 hours of preparation**

## Assay Procedure

1. Pipette 50 µL of either samples or standards into duplicate wells in the plate.
2. Pipette 50 µL of Assay Buffer into duplicate wells of the Zero standard.
3. Pipette 75 µL of Assay Buffer into duplicate wells of the nonspecific binding (NSB).
4. Add 25 µL of 8-OHdG Conjugate to each well, except the NSB wells, using a repeater pipette.
5. Add 25 µL of 8-OHdG Antibody to each well using a repeater pipette.
6. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
7. Cover the plate with the plate sealer and shake at room temperature for 2 hours. If the plate is not shaken, signals will be approximately 15-20 % lower.
8. Aspirate the plate and wash each well 4 times with 300 µL Wash Buffer.
9. Tap the plate on clean absorbent towels to dry.
10. Add 100 µL of TMB Substrate to each well using a multichannel/repeater pipette.
11. Incubate at room temperature for 30 minutes.
12. Add 50 µL of Stop Solution to each well using a multichannel/repeater pipette.
13. Read the optical density at 450 nm.

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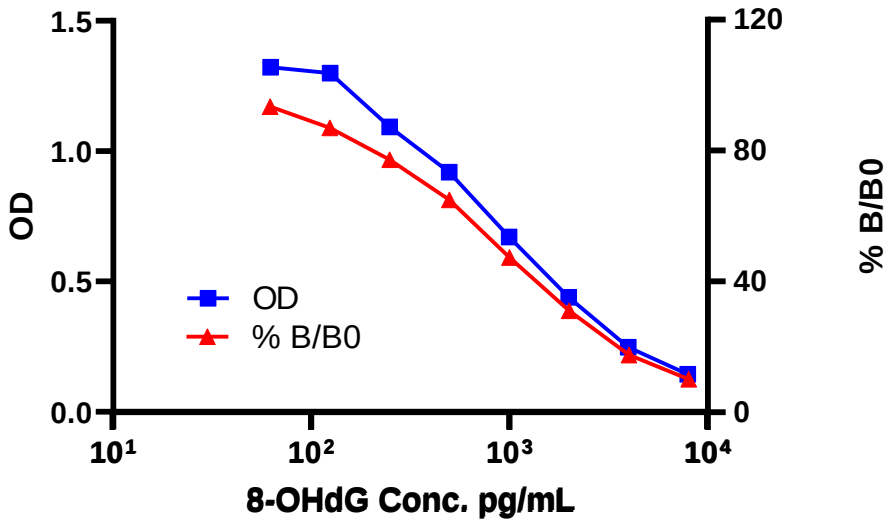
## Calculation

- Average the duplicate optical density (OD) readings for each standard and sample.
- Subtract the mean ODs for the NSB from all OD values
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader
- Calculate the %B/B0 ratio.
  - **Note:** B0 is the binding for the zero standard or the maximum binding well, which represents the maximum signal from enzyme captured by the specific antibody in competitive ELISA. All other standards and samples are expressed as a percentage of this value (%B/B0).
- The concentrations obtained should be multiplied by the dilution factor to obtain sample values.

**Conversion Factor:** 100 pg/mL of 8-OHdG is equivalent to 353.0 pM




A typical standard curve of ZellX® 8-OHdG Assay kit

**Run your own standard curves for calculation of results**

## Assay range

The limit of detection of ZellX® 8-OHdG assay was determined as 82.2 pg/mL.

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## Sensitivity

The sensitivity of the ZellX® 8-OHdG assay was determined as 50.9 pg/mL.

## Precision

Intra-Assay Precision (Precision within an assay): 3 human serum samples were tested 20 times in an assay.

Inter-Assay Precision (Precision between assays): 3 human serum samples were tested in duplicate on 19 different assays over multiple days.

| *Item* | *%CV* |
|---|---|
| **Intra assay** | 8.2, 11.7, 7.1 |
| **Inter assay** | 13.4, 8.1, 8.3 |

## Cross Reactivity

The following cross reactants were tested in the assay and calculated at the 50% binding point.

| *Steroid* | *Cross Reactivity (%)* |
|---|---|
| **8-Hydroxy-2'-deoxyguanosine** | 100% |
| **8-Hydroxyguanosine** | 27.32 |
| **8-Hydroxyguanine** | 9.50 |

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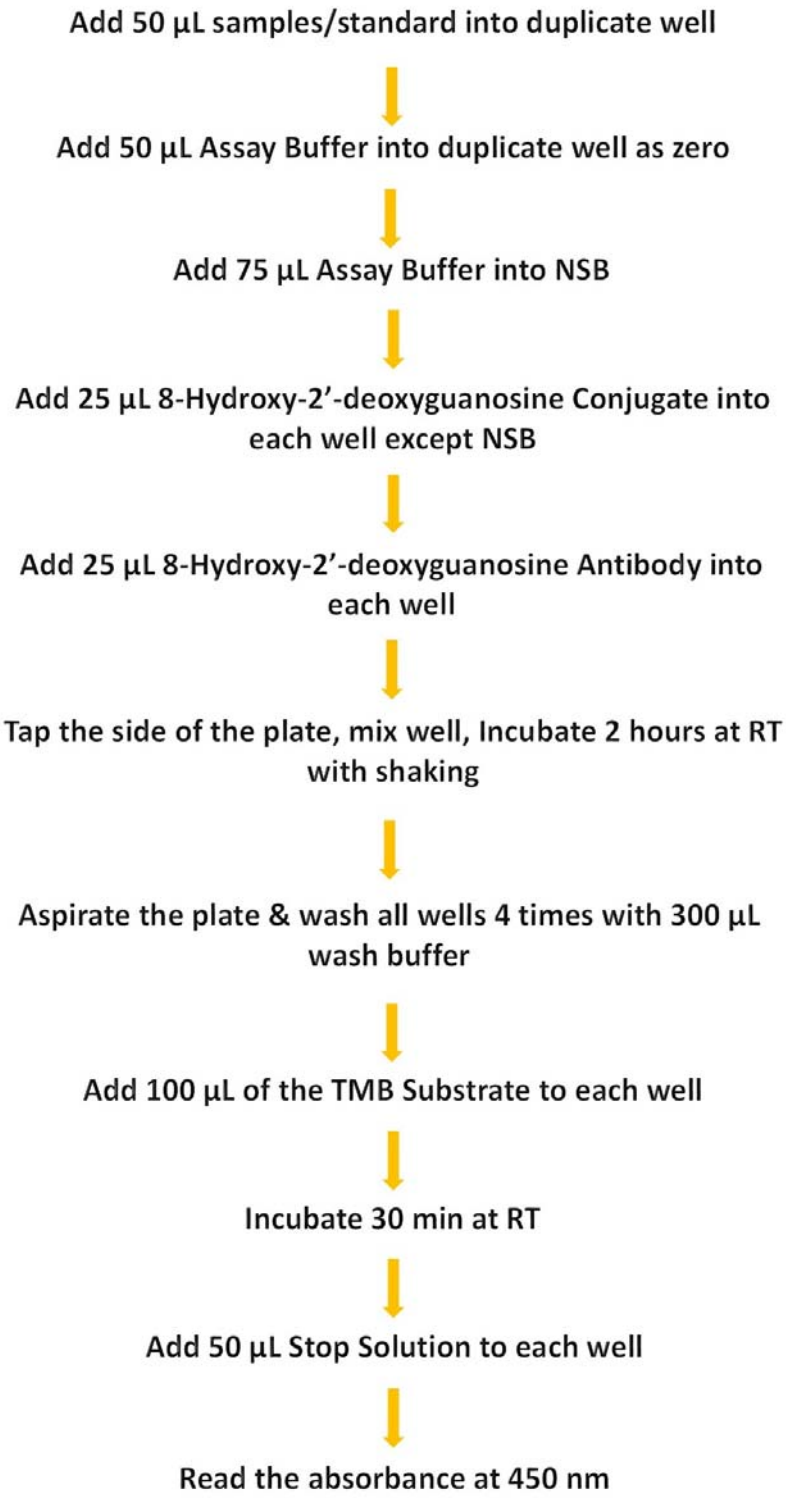
## Protocol summary

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