Product Manual

OxiSelect™ Nitrosative DNA/RNA Damage ELISA Kit (8-Nitroguanine Quantitation)

Catalog Number

STA-825	96 assays
STA-825-5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

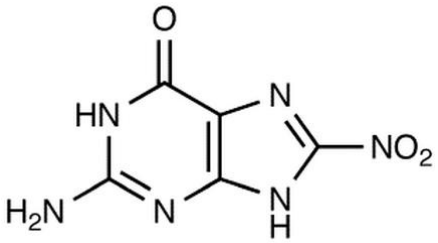
CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Free radicals and other reactive species are constantly generated *in vivo* and such reactive oxygen species (ROS) or reactive nitrogen species (RNS) cause damage to biomolecules, a process held in check only by the existence of multiple antioxidant and repair systems as well as the replacement of damaged nucleic acids, proteins and lipids. DNA and RNA are probably the most biologically significant target of oxidative or nitrosative attack, and it is widely thought that continuous ROS or RNS damage to DNA/RNA is a significant contributor to the age-related development of the major inflammation related diseases and cancers, such as those of the colon, breast, rectum, and prostate. Among numerous types of nitrosative DNA/RNA damage, the formation of 8-nitroguanine (8-NO_2-Gua) is a ubiquitous marker of RNS stress. Different RNS such as peroxynitrite or nitrogen oxides formed during pathophysiological conditions can nitrate guanine and related nucleosides and nucleotides in free form or embedded in DNA/RNA.

8-NO_2-Gua, one of these nitrosative DNA/RNA damage byproducts, is physiologically formed and propagated by chronic infection and inflammation which is ultimately associated with malignancies. It is predominantly found in inflammatory cells or epithelial cells in inflamed tissues. It has been shown to act as a pro-oxidant by stimulating superoxide and inducible nitric oxide synthase activity (iNOS). This excess nitric oxide (NO) production is important to this inflammation process which propagates 8-NO_2-Gua. Once formed, 8-nitroguanine is unstable within the DNA structure with a half-life of about 4 hrs after which it depurinates to release free 8-nitroguanine. This results in mutagenic abasic sites that can facilitate the creation of G:C→T:A transversions.

The OxiSelect™ Nitrosative DNA/RNA Damage ELISA Kit is a competitive enzyme immunoassay developed for rapid detection and quantitation of 8-nitroguanine in urine, serum, or plasma samples. The quantity of 8-nitroguanine in unknown samples is determined by comparing its absorbance with that of a known 8-nitroguanine standard curve. The kit has an 8-nitroguanine detection sensitivity of approximately 4 ng/mL. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown samples.



8-Nitroguanine

Assay Principle

The OxiSelect™ Nitrosative DNA/RNA Damage ELISA kit is a competitive ELISA for the quantitative measurement of 8-nitroguanine (8-NO_2-Gua). The unknown 8-NO_2-Gua samples or 8-NO_2-Gua standards are first added to an 8-NO_2-Gua-BSA conjugate preabsorbed microplate. After a brief incubation, an anti-8-NO_2-Gua monoclonal antibody is added, followed by an HRP conjugated

secondary antibody. The 8-NO_2-Gua content in unknown samples is determined by comparison with predetermined 8-NO_2-Gua standard curve.

Related Products

1. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
2. STA-321: OxiSelect™ DNA Double-Strand Break (DSB) Staining Kit
3. STA-322: OxiSelect™ UV-induced DNA Damage ELISA Kit (CPD Quantitation)
4. STA-323: OxiSelect™ UV-induced DNA Damage ELISA Kit (6-4PP Quantitation)
5. STA-324: OxiSelect™ Oxidative DNA Damage Quantitation Kit (AP sites)
6. STA-325: OxiSelect™ Oxidative RNA Damage ELISA Kit (8-OHG Quantitation)
7. STA-350: OxiSelect™ Comet Assay Kit (3-Well Slides), 15 Assays
8. STA-357: OxiSelect™ BPDE DNA Adduct ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. 96-well Protein Binding Plate (Part No. 231001): One strip-well 96 well microplate.
2. Anti-8-Nitroguanine Antibody (Part No. 282501): One 10 µL vial of anti-8-Nitroguanine.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 230003): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.
8. 8-Nitroguanine Standard (Part No. 282502): One 10 µL vial of 1 mg/mL 8-NO_2-Gua in DMSO.

Box 2 (shipped on blue ice packs)

1. 8-Nitroguanine Conjugate (Part No. 282503): One 25 µL vial of 8-NO_2-Gua-BSA conjugate in PBS.

Materials Not Supplied

1. 8-Nitroguanine samples such as urine, serum, or plasma
2. 1X PBS
3. 10 kDa molecular weight cutoff (MWCO) centrifuge spin filter (e.g. Amicon Ultra 0.5mL)
4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips

5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
6. Multichannel micropipette reservoir
7. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the 8-Nitroguanine Standard at -20ºC and the 8-Nitroguanine Conjugate at -80ºC to avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

Preparation of Reagents

- 8-Nitroguanine Coated Plate: Dilute the proper amount of 8-Nitroguanine Conjugate 1:400 in 1X PBS depending on the number of required assays. (Example: Add 10 µL of 8-Nitroguanine Conjugate stock tube to 3.990 µL 1X PBS to coat 40 wells). Add 100 µL of this 8-Nitroguanine Conjugate solution to each well and incubate overnight at 4ºC. Remove the 8-Nitroguanine coating solution and wash once with 1X PBS. Blot plate on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1-2 hr at room temperature. Transfer the plate to 4ºC and remove the Assay Diluent immediately before use.

  Note: The 8-Nitroguanine-conjugate coated wells are not stable and should be used within 24 hrs after coating. Only coat the number of wells to be used immediately.

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-8-Nitroguanine Antibody and Secondary Antibody: Immediately before use dilute the Anti-8-Nitroguanine Antibody 1:2000 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

1. Prepare fresh standards by diluting the 8-Nitroguanine Standard stock from 1 mg/mL to 1 µg/mL in Assay Diluent for a 1:1000 final dilution. (Example: Add 2 µL of 8-Nitroguanine Standard stock tube to 1.998 mL of Assay Diluent).
2. Prepare a dilution series of 8-Nitroguanine standards in the concentration range of 0 ng/mL to 1000 ng/mL by diluting the 8-Nitroguanine Standard in Assay Diluent (Table 1).

Standard Tubes	8-Nitroguanine Standard (µL)	Assay Diluent (µL)	8-Nitroguanine (ng/mL)
1	2	1998	1000
2	500 of Tube #1	500	500
3	500 of Tube #2	500	250
4	500 of Tube #3	500	125
5	500 of Tube #4	500	62.5
6	500 of Tube #5	500	31.3
7	500 of Tube #6	500	15.6
8	500 of Tube #7	500	7.8
9	500 of Tube #8	500	3.9
10	0	500	0

Table 1. Preparation of 8-Nitroguanine Standards

Preparation of Samples

Clear urine, plasma or serum samples can be used directly in the assay or diluted in either Assay Diluent or 1X PBS. Samples containing precipitates should be centrifuged at 3000 g for 10 minutes, or filtered through 0.45 µm filter, prior to use in the assay.

Note: For mouse or rat serum or plasma samples it is highly recommended to filter the sample with a 10kDa spin filter prior to testing.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each 8-Nitroguanine sample including unknown and standard should be assayed in duplicate.
2. Add 50 µL of unknown sample or 8-Nitroguanine standard to the wells of the 8-Nitroguanine Conjugate coated plate. Incubate at room temperature for 10 minutes on an orbital shaker.
3. Add 50 µL of the diluted anti-8-Nitroguanine antibody to each well, incubate at room temperature for 1 hour on an orbital shaker.
4. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Secondary Antibody, HRP Conjugate to all wells.
6. Incubate at room temperature for 1 hour on an orbital shaker.
7. Wash microwell strips 3 times according to step 4 above. Proceed immediately to the next step.

8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical Nitrosative DNA/RNA Damage ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

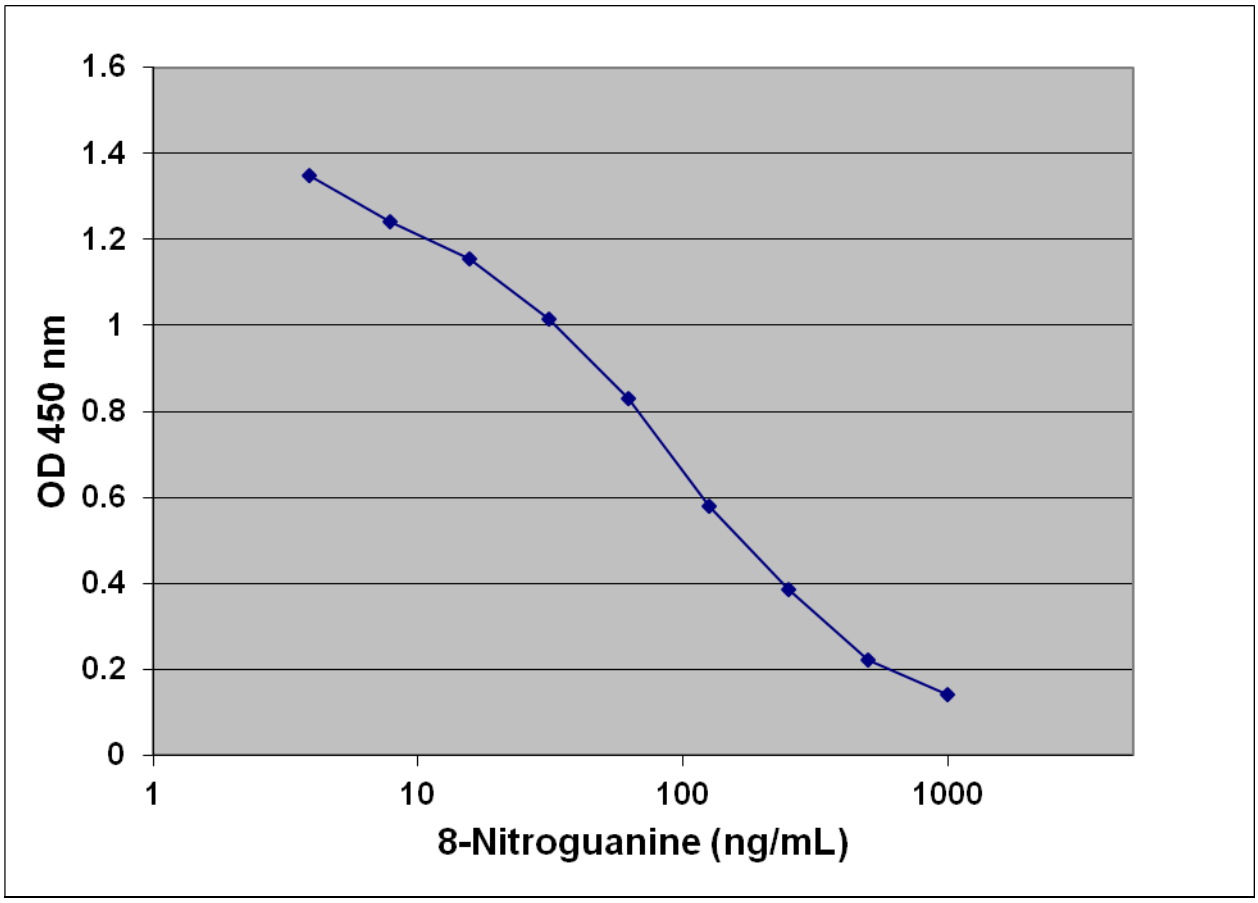


Figure 1: 8-Nitroguanine ELISA Standard Curve.

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3. Pinlaor, S., et al. (2004) Carcinogenesis **25**, 1535-1542.

Recent Product Citations

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6. Phookphan, P. et al. (2017). Hypomethylation of inflammatory genes (COX2, EGR1, and SOCS3) and increased urinary 8-nitroguanine in arsenic-exposed newborns and children. *Toxicol Appl Pharmacol*. **316**:36-47. doi: 10.1016/j.taap.2016.12.015.

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