Product Manual

OxiSelect™ Oxidative DNA Damage Quantitation Kit (AP Sites)

Catalog Number

STA-324 **50 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Introduction

Free radicals and other reactive species are constantly generated *in vivo* and cause oxidative 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 lipids and proteins. DNA is probably the most biologically significant target of oxidative attack, and it is widely thought that continuous oxidative damage to DNA is a significant contributor to the age-related development of the major cancers, such as those of the colon, breast, rectum, and prostate. Among numerous types of oxidative DNA damage, apurinic/apyrimidinic (AP or abasic) site is one of the prevalent lesions of oxidative DNA damage. Abasic sites arise in DNA at a significant rate by spontaneous base loss as in depurination, by DNA oxidation, or by the action of DNA glycosylases. Estimates of the number of abasic sites generated per mammalian cell run as high as 50,000 to 200,000 per day. Unrepaired abasic sites inhibit topoisomerases, replication, and transcription and can be mutagenic because of bypass synthesis on nontemplated DNA.

The OxiSelect™ Oxidative DNA Damage Quantitation Kit (AP sites) uses an Aldehyde Reactive Probe (ARP) to react specifically with an aldehyde group on the open ring form of AP sites. This allows for the AP sites to be tagged with biotin which is later detected with Streptavidin-Enzyme conjugate. The quantity of AP sites in unknown DNA sample is determined by comparing its absorbance with a standard curve generated from the provided DNA standard containing predetermined AP sites. The kit has a detection sensitivity range of 4 to 40 AP sites per 1 x 10^5 bp. Each kit provides sufficient reagents to perform up to 50 assays for unknown samples, excluding the 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-325: OxiSelect™ Oxidative RNA Damage ELISA Kit (8-OHG Quantitation)
4. STA-351: OxiSelect™ Comet Assay Kit (3-Well Slides), 75 Assays
5. STA-355: OxiSelect™ 96-Well Comet Assay Kit

Kit Components

1. Glycogen Solution (Part No. 232401): One 100 µL vial of 10 mg/mL glycogen.
2. Sodium Acetate Solution (Part No. 232402): One 1.0 mL vial of 3M Sodium Acetate, pH 5.5.
3. ARP Solution (Part No. 232403): One 250 µL vial of 10 mM ARP.
4. DNA High-Binding Plate (Part No. 232404): One 96-well strip plate.
5. DNA Binding Solution (Part No. 232405): One 6 mL bottle.
6. 10X Wash Buffer (Part No. 232406): One 30 mL bottle.
7. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
8. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
9. Stop Solution (Part. No. 310808): One 12 mL bottle.
10. Reduced DNA Standard (Part No. 232407): One 1.0 mL vial of 6 µg/mL fully reduced in TE Buffer (0 ARP/100,000 bp).

11. ARP-DNA Standard (Part No. 232408): One 400 µL vial of 6 µg/mL ARP-DNA in TE Buffer (40 ARP/100,000 bp).

Materials Not Supplied

1. DNA samples from cell or tissue for measuring DNA damage
2. TE Buffer: 10 mM Tris, pH 7.5, 1 mM EDTA
3. 100% and 70% Ethanol

Storage

Upon receipt, aliquot and store both the Reduced DNA and ARP-DNA Standards at -20ºC to avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Streptavidin-Enzyme Conjugate: Immediately before use, dilute the Streptavidin-Enzyme Conjugate 1:1000 with 1X Wash Buffer. Do not store diluted solutions.

Preparation of Standard Curve

Prepare a dilution series of ARP-DNA standards in the concentration range of 0 – 40 ARP/100,000 bp according to Table 1.

Tubes	ARP-DNA Standard (µL)	Reduced DNA Standard (µL)	TE Buffer (µL)	Total Volume (µL)	DNA Concentration (µg/mL)	AP Sites per 100,000 bp
1	20	0	100	120	1	40
2	16	4	100	120	1	32
3	12	8	100	120	1	24
4	8	12	100	120	1	16
5	4	16	100	120	1	8
6	2	18	100	120	1	4
7	1	19	100	120	1	2
8	0	20	100	120	1	0

Table 1. Preparation of ARP-DNA Standards

Assay Protocol

I. ARP Reaction

1. Isolate genomic DNA with desired method and dissolve the genomic DNA in TE buffer. Dilute the genomic DNA with TE buffer to 100 µg/mL.

Note: During DNA extraction, avoid heating the DNA solution, or any procedure will introduce AP sites. We recommend using DNAZOL reagent to extract DNA and dissolve DNA in TE buffer.

2. Mix 5 µL of purified genomic DNA (100 µg/mL) with 5 µL of ARP solution in a microcentrifuge tube and incubate 1 hr at 37ºC.

3. Add 90 µL of TE buffer and 1 µL of Glycogen Solution to each tube and mix well.
4. Add 10 µL of Sodium Acetate Solution to each tube, mix well.
5. Add 300 µL of absolute ethanol to each tube and mix well and incubate at -20ºC for 30 minutes.
6. Centrifuge for 10-20 minutes at 14,000 g and carefully wash the pellet three times with 70% ethanol.
7. Dissolve the DNA pellet in 10-50 µL of TE buffer and determine the DNA concentration with desired method. ARP-derived DNA can be stored at -20ºC for up to one year.

Note: It is important that the ARP-derived DNA concentration is determined precisely for the accurate measurement of AP sites. We recommend using Invitrogen's Quanti-iT™ DNA assay kit to measure DNA concentration.

II. Determination of AP sites in DNA:

1. Dilute the ARP-derived DNA sample to 1 µg/mL with TE buffer.
2. Add 50 µL of ARP-derived DNA sample or each dilution of the prepared ARP-DNA standards to the DNA High-binding plate. Add 50 µL of DNA Binding Solution to each well. Mix well by pipetting and incubate at room temperature for 2 hrs or overnight on an orbital shaker. Each sample including unknown and standard should be assayed in duplicate.
3. 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.
4. Add 100 µL of diluted Streptavidin-Enzyme Conjugate to each well and incubate at 37ºC for 1 hr.
5. 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.
6. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature for 5 to 20 minutes on an orbital shaker.
7. Stop the enzymatic 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).
8. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

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

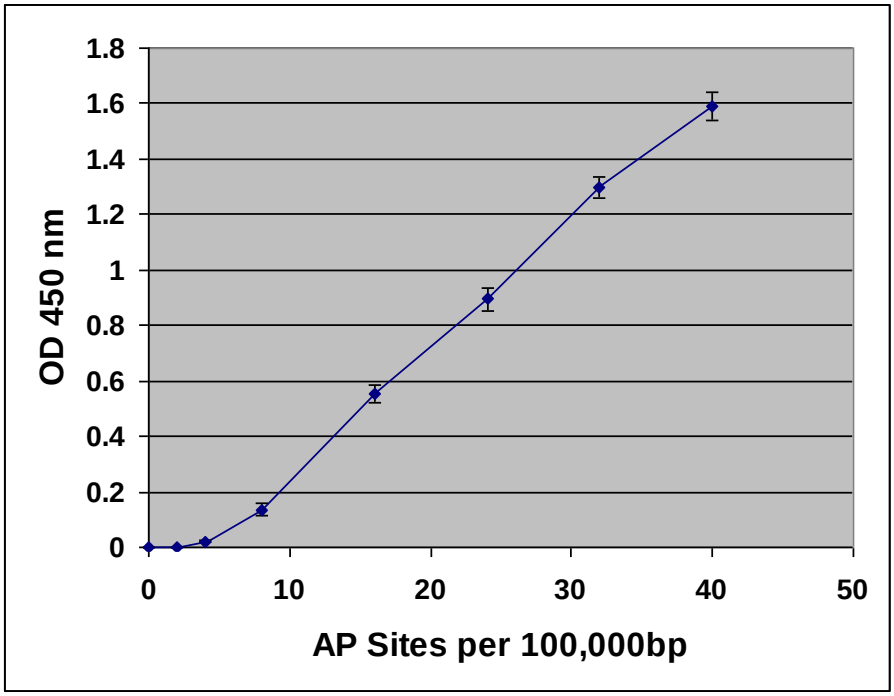

Figure 1: ARP-DNA Standard Curve.

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Recent Product Citations

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