NOTE: Revision to Assay Protocol

Product Manual

OxiSelect™ Aldehyde-Induced DNA Damage ELISA Combo Kit (Ethenoadenosine / Ethenocytidine Quantitation)

Catalog Number

STA-820-C 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Oxidation of phospholipids can lead to the formation of lipid hydroperoxides. These resulting short-lived hydroperoxides can either be converted to inert fatty acid alcohols, or can react with metals to form aldehydes such as malondialdehyde (MDA), 4-hydroxynonenal (HNE), acrolein, and crotonaldehyde. These aldehydes (which can also be formed through exposure to carcinogenic substances such as urethane or vinyl chloride) can damage DNA resulting in the formation of 4 major etheno adducts: 1,N^6-ethenodeoxyadenosine, 3,N^4-ethenodeoxycytidine, N^2,3-ethenodeoxyguanosine, and 1,N^2-ethenodeoxyguanosine. The presence of etheno bases mainly leads to base pair substitution mutations. The 1,N^6-ethenodeoxyadenosine base can cause AT to TA or AT to CG transversions, as well as AT to GC transitions, while the 3,N^4-ethenodeoxycytidine base can cause CG to TA transitions as well as CG to AT transversions. The level of etheno damage has been shown to increase during conditions of oxidative stress, such as in the presence of nitric oxide overproduction.

Cell Biolabs' OxiSelect™ Aldehyde-Induced DNA Damage ELISA Combo Kit (Ethenoadenosine / Ethenocytidine Quantitation) is a competitive enzyme immunoassay developed for rapid detection and quantitation of Ethenoadenosine or Ethenocytidine in any DNA samples. The quantity of ethenoadenosine or ethenocytidine in an unknown sample is determined by comparing its absorbance respectively with that of a known ethenoadenosine or ethenocytidine standard curve. Ethenoadenosine or ethenocytidine are assayed individually in separate wells, and each kit provides sufficient reagents to perform up to a total of 96 assays for ethenoadenosine and/or ethenocytidine combined in any ratio (e.g. 64 wells for ethenoadenosine and 32 wells for ethenocytidine, or 48 wells for each target).

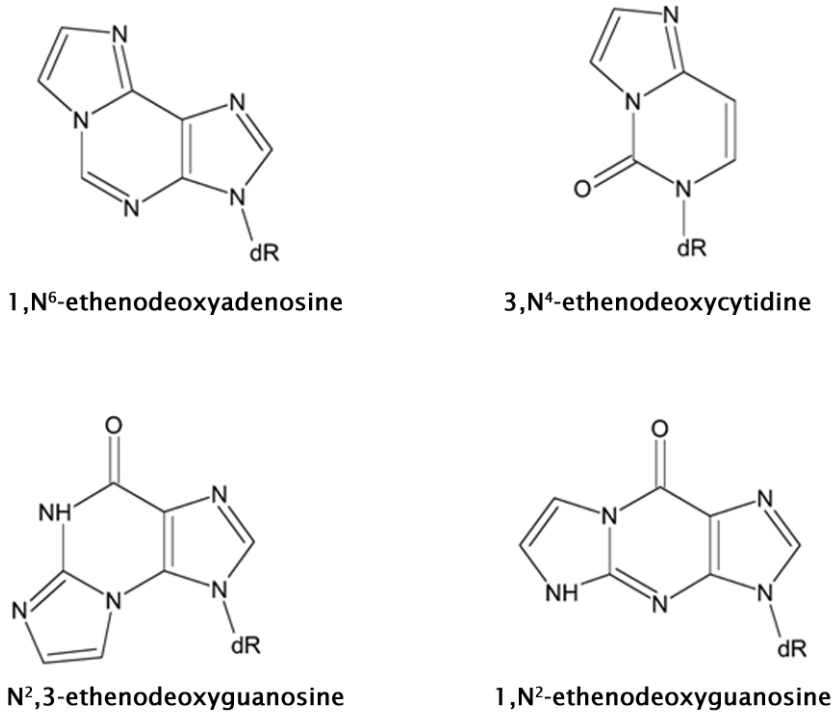

Figure 1: Structures of DNA Lesions Induced by Aldehydes.

Assay Principle

The OxiSelect™ Aldehyde-Induced DNA Damage ELISA Combo Kit is a competitive ELISA for the quantitative measurement of ethenoadenosine or ethenocytidine. The unknown damaged DNA samples or etheno base standards are first added to an etheno-damaged DNA preabsorbed microplate. After a brief incubation, an anti-ethenoadenosine or anti-ethenocytidine monoclonal antibody is added, followed by an HRP conjugated secondary antibody. The etheno base content in unknown samples is determined by comparison with predetermined ethenoadenosine or ethenocytidine standard curves.

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-351: OxiSelect™ Comet Assay Kit (3-Well Slides), 75 Assays
8. STA-355: OxiSelect™ 96-Well Comet Assay Kit
9. STA-357: OxiSelect™ BPDE DNA Adduct ELISA Kit
10. STA-380: Global DNA Methylation ELISA Kit

Kit Components

1. DNA High-Binding Plate (Part No. 232404): One 96-well strip plate.
2. DNA Binding Solution (Part No. 232405): One 6 mL bottle.
3. 100X Etheno DNA Conjugate (Part No. 282001): One 50 µL vial.
4. Anti-Ethenoadenosine Antibody (Part No. 282002): One 10 µL vial of anti-ethenoadenosine.
5. Anti-Ethenocytidine Antibody (Part No. 282101): One 10 µL vial of anti-ethenocytidine.
6. Secondary Antibody, HRP Conjugate (Part No. 230003): One 20 µL vial.
7. Assay Diluent (Part No. 310804): One 50 mL bottle.
8. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
9. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
10. Stop Solution (Part. No. 310808): One 12 mL bottle.
11. Ethenoadenosine Standard (Part No. 282003): One 100 µL vial of 100 µM ethenoadenosine in 1X TE Buffer.
12. Ethenocytidine Standard (Part No. 282102): One 100 µL vial of 1 mM ethenocytidine in 1X TE Buffer.

Materials Not Supplied

1. DNA samples such as cell or tissue genomic DNA
2. DNA Extraction Kit
3. 1X TE Buffer (10 mM Tris, pH 8.0, 1 mM EDTA)
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, store the Ethenoadenosine Standard, Ethenocytidine Standard, 100X Etheno DNA Conjugate, Anti-Ethenoadenosine Antibody and Anti-Ethenocytidine Antibody at -20ºC. Store all other components at 4ºC.

Preparation of Reagents

- Etheno DNA Conjugate Coated Wells:

  Note: The Etheno DNA 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.

  1. Dilute 100X Etheno DNA Conjugate 1:100 in 1X TE Buffer. For example, for 10 wells add 5 µL of Etheno DNA conjugate to 495 µL of 1X TE and mix well.
  2. Add 50 µL of diluted Etheno DNA conjugate to the wells.
  3. Add 50 µL of DNA Binding solution to each well and **incubate overnight at room temperature**.
  4. Remove the Etheno Conjugate/DNA Binding solution and add 200 µL of Assay Diluent to each well.
  5. Incubate for 1 hr at room temperature. Transfer the plate to 4ºC until ready to begin the assay.

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-Ethenoadenosine Antibody, Anti-Ethenocytidine Antibody and secondary antibody: Immediately before use dilute the Anti-Ethenoadenosine Antibody or Anti-Ethenocytidine Antibody 1:500 with Assay Diluent. Immediately before use dilute the secondary antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curves

1. Prepare a dilution series of Ethenoadenosine standards in the concentration range of 0 – 1000 nM by diluting the standard in Assay Diluent according to Table 1 below.

Standard Tubes	100 µM Ethenoadenosine Standard (µL)	Assay Diluent (µL)	Ethenoadenosine (nM)
1	10	990	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	0	500	0

Table 1. Preparation of Ethenoadenosine Standards.

2. Prepare a dilution series of Ethenocytidine standards in the concentration range of 0 – 10 µM by diluting the standard in Assay Diluent according to Table 2 below.

Standard Tubes	1 mM Ethenocytidine Standard (µL)	Assay Diluent (µL)	Ethenocytidine (µM)
1	10	990	10
2	500 of Tube #1	500	5
3	500 of Tube #2	500	2.5
4	500 of Tube #3	500	1.25
5	500 of Tube #4	500	0.625
6	500 of Tube #5	500	0.313
7	500 of Tube #6	500	0.156
8	0	500	0

Table 2. Preparation of Ethenocytidine Standards.

Assay Protocol

Note: Ethenoadenosine and ethenocytidine are assayed in separate wells, but any well in the Etheno DNA Coated Plate may be used for either target.

1. Extract DNA from cell or tissue samples using a commercial DNA Extraction kit or other desired method.
2. Add 50 µL of unknown DNA sample, Ethenoadenosine Standard, or Ethenocytidine Standard to the Etheno Conjugate Coated wells of the plate (see Preparation of Reagents section). Each DNA sample including unknown and standard should be assayed in duplicate.
3. Add 50 µL of diluted Anti-Ethenoadenosine or Anti-Ethenocytidine Antibody (see Preparation of Reagents section) to each tested 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 each 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 each well. Incubate at room temperature for 1 hour on an orbital shaker. During this incubation, warm Substrate Solution to room temperature.
6. Wash the strip wells 3 times according to step 5 above. Proceed immediately to the next step.
7. 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.
8. 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).
9. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wavelength.

Example of Results

The following figures demonstrate typical OxiSelect™ Aldehyde-Induced DNA Damage ELISA Combo Kit (Ethenoadenosine/Ethenocytidine Quantitation) results. One should use the data below for reference only. This data should not be used to interpret actual results.

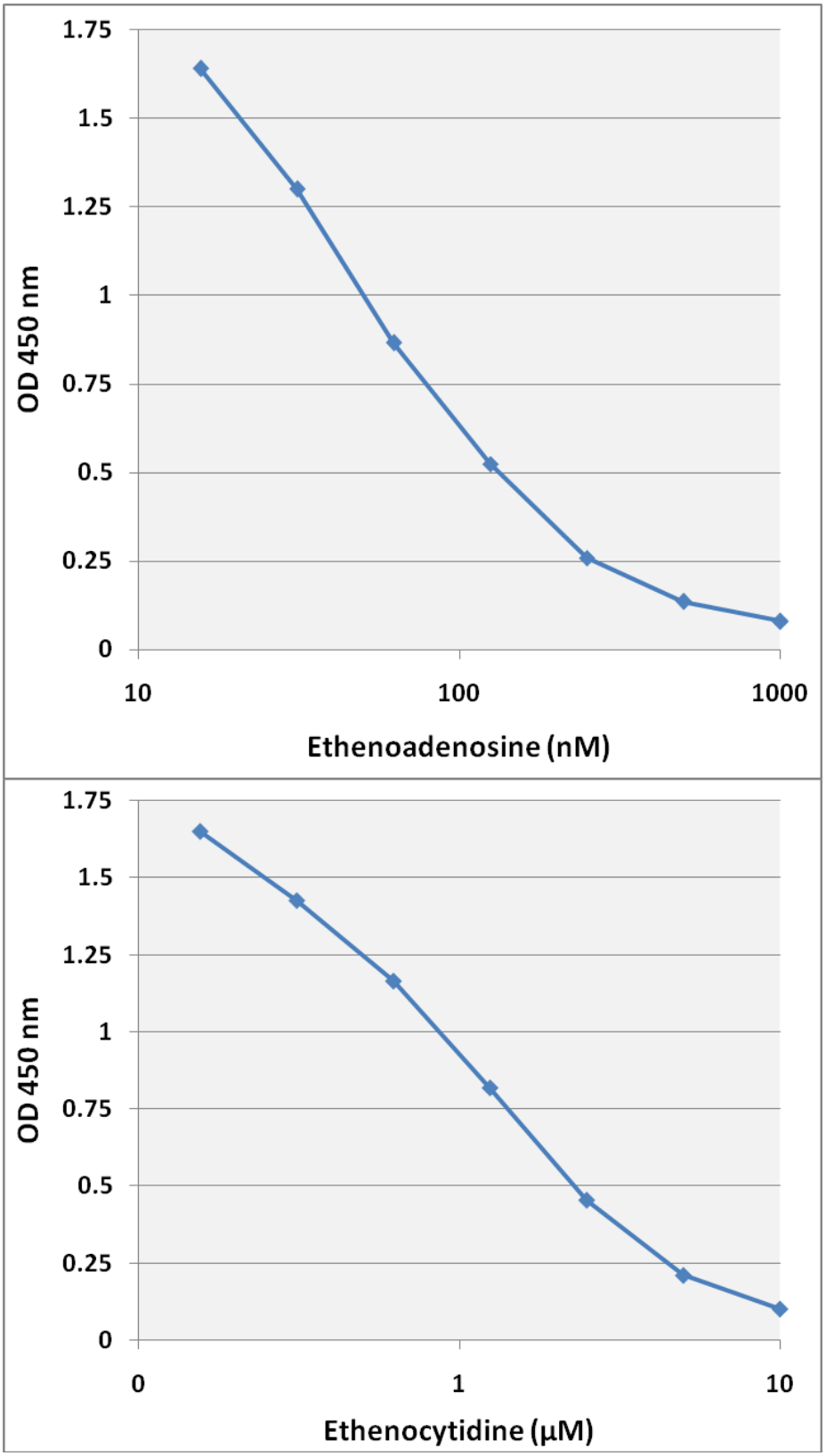

Figure 2: Ethenoadenosine and Ethenocytidine Standard Curves.

References

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Warranty

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Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com