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User's Manual

Aldehyde-Induced DNA Damage ELISA Kit (Ethenocytidine Quantitation)

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

CD' Aldehyde-Induced DNA Damage ELISA Kit (Ethenocytidine Quantitation) is a competitive enzyme immunoassay developed for rapid detection and quantitation of ethenocytidine in any DNA sample. The quantity of ethenocytidine in an unknown sample is determined by comparing its absorbance respectively with that of a known Ethenocytidine standard curve.

General Description

Oxidation of phospholipids can lead to the formation of lipid hydroperoxides. These resulting shortlived 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,N6-ethenodeoxyadenosine, 3,N4-ethenodeoxycytidine, N2,3-ethenodeoxyguanosine, and 1,N2-ethenodeoxyguanosine. The presence of etheno bases mainly lead to base pair substitution mutations. The 1,N6-ethenodeoxyadenosine base can cause AT to TA or AT to CG transversions, as well as AT to GC transitions, while the 3,N4-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.

Principles of Testing

The Aldehyde-Induced DNA Damage ELISA Kit is a competitive ELISA for the quantitative measurement of 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-Ethenocytidine monoclonal antibody is added, followed by an HRP conjugated secondary antibody. The etheno base content in unknown samples is determined by comparison with a predetermined ethenocytidine standard curve.

Reagents And Materials Provided

1. 96-well Etheno DNA Coated Plate: One strip well 96-well plate.
2. Anti-Ethenocytidine Antibody: One 10 µL vial of anti-ethenocytidine.
3. Secondary Antibody, HRP Conjugate: One 50 µL vial.
4. Assay Diluent: One 50 mL bottle.
5. 10X Wash Buffer: One 100 mL bottle.
6. Substrate Solution: One 12 mL amber bottle.
7. Stop Solution: One 12 mL bottle.
8. Ethenocytidine Standard: One 100 µL vial of 4 mM Ethenocytidine in 1X TE Buffer.

Materials Required But Not Supplied

1. DNA samples such as cell or tissue genomic DNA
2. DNA Extraction Kit
3. PBS
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 Ethenocytidine Standard and Anti-Ethenocytidine Antibody at -20°C. Store all other components at 4°C until their expiration dates.

Reagent Preparation

1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.

Anti-Ethenocytidine Antibody and Secondary Antibody, HRP Conjugate: Immediately before use dilute the 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.

Assay Procedure

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 or Ethenocytidine Standard to the wells of the plate. Each DNA sample including unknown and standard should be assayed in duplicate.
3. Add 50 µL of diluted 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 wave length.

Typical Standard Curve

Preparation of Standard Curves

Dilute the Ethenocytidine Standard 100 fold to 40 µM in Assay Diluent. For example, add 10 µL of the 4 mM Ethenocytidine Standard to 990 µL of Assay Diluent. Prepare a dilution series of Ethenocytidine standards in the concentration range of 0 - 40 µM by diluting the standard in Assay Diluent according to Table 2 below.

Standard Tubes	4 mM Ethenocytidine Standard (µL)	Assay Diluent (µL)	Ethenocytidine (µM)
1	10	990	40
2	500 of Tube #1	500	20
3	500 of Tube #2	500	10
4	500 of Tube #3	500	5
5	500 of Tube #4	500	2.5
6	500 of Tube #5	500	1.25
7	500 of Tube #6	500	0.625
8	0	500	0

Table 1. Preparation of Ethenocytidine Standards.

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

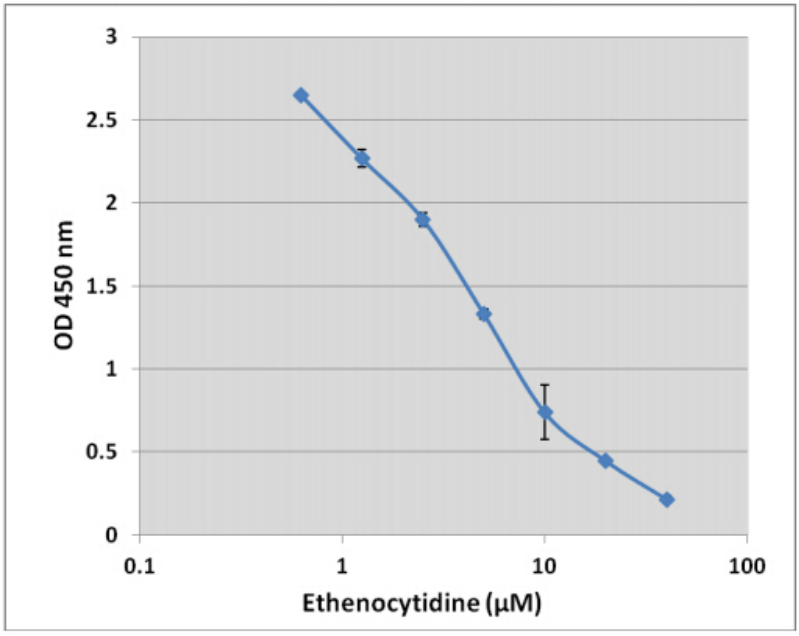

Figure 2: Ethenocytidine Standard Curve.