Datasheet for ABIN2345020

BPDE DNA Adduct ELISA Kit

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Overview

Quantity:	96 tests
Target:	BPDE DNA Adduct
Reactivity:	Others
Method Type:	DNA-Binding ELISA
Application:	ELISA

Product Details

Brand:	OxiSelect™
Sample Type:	DNA samples
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Characteristics:	The OxiSelect™ BPDE DNA Adduct ELISA Kit is an enzyme immunoassay developed for rapid detection of BPDE-DNA adducts. The quantity of BPDE adduct in DNA samples is determined by relative comparison of a known BPDE-DNA standard curve. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown protein samples.
Components:	1. DNA High-Binding Plate : One 96-well strip plate. 2. DNA Binding Solution : One 6 mL bottle. 3. Anti-BPDE Antibody (1000X) : One 20 µL vial of anti-BPDE-I antibody. 4. Secondary Antibody, HRP Conjugate (1000X) : One 50 µL vial. 5. Assay Diluent : One 50 mL bottle. 6. 10X Wash Buffer : One 100 mL bottle. 7. Substrate Solution : One 12 mL amber bottle. 8. Stop Solution (Part. No. 310808): One 12 mL bottle.

Product Details

	9. Reduced DNA Standard : One 200 µL vial of 0.2 mg/mL reduced DNA in TE Buffer. Box 2 (shipped on blue ice packs)
Material not included:	1. DNA samples such as cell or tissue genomic DNA 2. DNA Extraction Kit 3. 1X 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) 3

Target Details

Target:	BPDE DNA Adduct
Background:	Polycyclic aromatic hydrocarbons (PAHs) are potent, ubiquitous atmospheric pollutants commonly associated with oil, coal, cigarette smoke, and automobile exhaust fumes. Some PAH compounds are also found in cooked foods (e.g. grilled meat, smoked fish) and have been identified as mutagenic and carcinogenic. The toxicity of some PAHs has been demonstrated to induce malignant tumors in animal models and is also commonly believed to significantly contribute to human cancers. One PAH compound, benzo(a)pyrene, is notable for being the first chemical carcinogen to be discovered. Benzo(a)pyrene is a five-ring PAH known to be a procarcinogen, its mechanism of carcinogenesis is dependent on a 3-step enzymatic metabolism (Fig. 1 below) to the final mutagen benzo(a)pyrene diol epoxide (BPDE). Very reactive, BPDE binds covalently to proteins, lipids, and DNA (guanine residues) to produce BPDE adducts. If left unrepaired, DNA adducts may lead to permanent mutations resulting in cell transformation and ultimately tumor development. Figure 1: Benzo(a)pyrene catalyzed to various metabolites by Cytochrome P450 enzymes (CYP) and epoxide hydrolase (EH), resulting in the final carcinogen BPDE.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• For detection and quantitation of benzo(a)pyrene diol epoxide (BPDE) DNA adducts • BPDE-DNA standard included • Suitable for use with DNA samples such as cell or tissue genomic DNA
Plate:	Uncoated

Application Details

Protocol:	BPDE-DNA standards or unknown DNA samples are adsorbed onto a 96-well DNA high-binding plate. The BPDE-DNA adducts present in the sample or standard are probed with an Anti-BPDE-I Antibody, followed by an HRP Conjugated Secondary Antibody. The BPDE-DNA adduct content in an unknown sample is determined by comparing with a standard curve that is prepared from predetermined BPDE-DNA standards.
Reagent Preparation:	1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity. Anti-BPDE-I Antibody and Secondary Antibody: Immediately before use dilute the Anti-BPDE-I antibody 1:1000 and 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. Dilute DNA samples to 4 µg/mL in 1X TE Buffer. Note: Samples with high concentrations of BPDE may be further diluted 2-4 fold in 4 µg/mL Reduced BSA. A titration may be performed to ensure the samples fall in the range of the standard curve. 3. Add 50 µL of unknown DNA samples or BPDE-DNA standards to the wells of the DNA High-Binding plate. 4. Add 50 µL of DNA Binding Solution to each well. Mix well by pipetting and incubate at room temperature overnight on an orbital shaker. Each DNA sample including unknown and standard should be assayed in duplicate. 5. Remove the DNA solutions and wash twice with PBS. Blot plate on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1 hour at room temperature. 4 6. Remove the Assay Diluent. Blot plate on paper towels to remove excess fluid. 7. Add 100 µL of the diluted Anti-BPDE-I Antibody to all wells and incubate for 1 hour at room temperature on an orbital shaker. 8. Wash 5 times with 250 µL of 1X Wash Buffer 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. 9. Add 100 µL of the diluted Secondary Antibody-HRP Conjugate to all wells and incubate for 1 hour at room temperature on an orbital shaker. Wash the strip wells 5 times according to step 8 above. 10. Warm Substrate Solution to room temperature. Add 100 L 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. 11. Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time). 12. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length. Use the Reduced DNA Standard as an absorbance blank.
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid multiple freeze/thaw cycles.
Storage:	4 °C/-20 °C
Storage Comment:	Upon receipt, aliquot and store the Reduced DNA and BPDE-DNA Standards at -20°C to avoid multiple freeze/thaw cycles. Store all other kit components at 4°C until their expiration dates. 3

Publications

Product cited in:

Peters, Wickstrom, Siciliano: "Do biomarkers of exposure and effect correlate with internal exposure to PAHs in swine?" in: **Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals**, Vol. 21, Issue 3, pp. 283-91, (2016) (PubMed).

Beranek, Fiala, Kremlacek, Andrys, Hamakova, Chmelarova, Palicka, Borska: "Genetic polymorphisms in biotransformation enzymes for benzo[a]pyrene and related levels of benzo[a]pyrene-7,8-diol-9,10-epoxide-DNA adducts in Goeckerman therapy." in: **Toxicology letters**, Vol. 255, pp. 47-51, (2016) (PubMed).

Coric, Simic, Pekmezovic, Basta-Jovanovic, Savic Radojevic, Radojevic-Skodric, Matic, Dragicevic, Radic, Bogdanovic, Dzamic, Pljesa-Ercegovac: "Combined GSTM1-Null, GSTT1-Active, GSTA1 Low-Activity and GSTP1-Variant Genotype Is Associated with Increased Risk of Clear Cell Renal Cell Carcinoma." in: **PLoS ONE**, Vol. 11, Issue 8, pp. e0160570, (2016) (PubMed).

Su, Zhao, Guo, Bin, Yang, Liu, Han, Niu, Ke, Wang, Geng, Jin, Dai, Lin: "Interaction of benzo[a]pyrene with other risk factors in hepatocellular carcinoma: a case-control study in Xiamen, China." in: **Annals of epidemiology**, Vol. 24, Issue 2, pp. 98-103, (2014) (PubMed).

Barhoumi, Mouneimne, Chapkin, Burghardt: "Effects of fatty acids on benzo[a]pyrene uptake and metabolism in human lung adenocarcinoma A549 cells." in: **PLoS ONE**, Vol. 9, Issue 3, pp. e90908, (2014) (PubMed).

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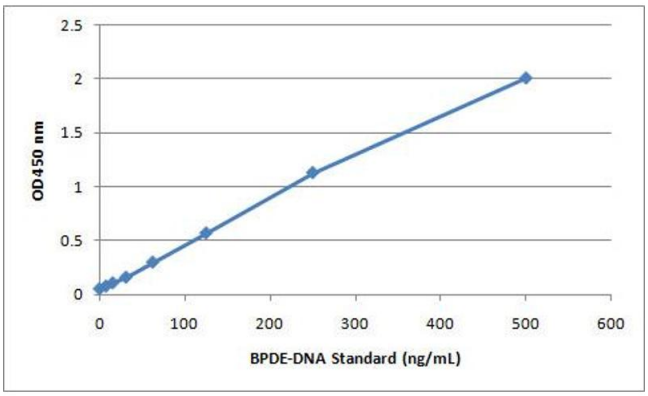

ELISA

Image 1. BPDE-DNA Standard Curve.