KAMIYA BIOMEDICAL COMPANY

BPDE DNA Adduct ELISA

For the rapid detection of BPDE-DNA adducts

Cat. No. KT-938

For Research Use Only. Not for use in diagnostic procedures.

Product Information
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INTRODUCTION
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.

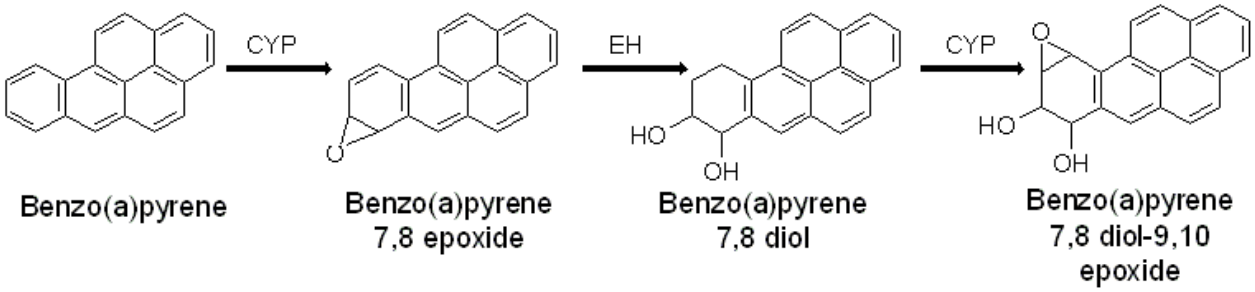

Figure 1: Benzo(a)pyrene catalyzed to various metabolites by Cytochrome P450 enzymes (CYP) and epoxide hydrolase (EH), resulting in the final carcinogen BPDE.

The BPDE DNA Adduct ELISA 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 calibration curve. Each kit provides sufficient reagents to perform up to 96 assays, including calibration curve and unknown protein samples.

ASSAY PRINCIPLE
BPDE-DNA calibrators or unknown DNA samples are adsorbed onto a 96-well DNA high-binding plate. The BPDE-DNA adducts present in the sample or calibrator 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 calibration curve that is prepared from predetermined BPDE-DNA calibrators.

COMPONENTS
1. DNA High-Binding Plate: One 96-well strip plate.

2. DNA Binding Solution: One 6 mL bottle.

3. Anti-BPDE Antibody (1,000X): One 20 µL vial of anti-BPDE-I antibody.

4. Secondary Antibody, HRP Conjugate (1,000X): 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: One 12 mL bottle.

9. Reduced DNA Calibrator: One 200 µL vial of 0.2 mg/mL reduced DNA in TE Buffer.

10. BPDE-DNA Calibrator: One 30 µL vial of 0.1 mg/mL BPDE-DNA in TE Buffer.

MATERIALS REQUIRED BUT 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 1,000 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 Reduced DNA and BPDE-DNA Calibrators at -20ºC to avoid multiple freeze/thaw cycles. Store all other kit components at 4ºC.

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 1:1,000 and Secondary Antibody 1:1,000 with Assay Diluent. Do not store diluted solutions.

CALIBRATION CURVE PREPARATION

1. Freshly prepare 4 µg/mL of Reduced DNA by diluting the 0.2 mg/mL stock 1:50 in 1X TE Buffer. Example: Add 40 µL to 1.96 mL of 1X TE Buffer.

2. Freshly prepare 4 µg/mL of BPDE-DNA by diluting the 0.1 mg/mL stock 1:25 in 1X TE Buffer. Example: Add 6 µL to 144 µL of 1X TE Buffer.

3. Prepare a series of BPDE-DNA calibrators according to Table 1.

Calibrator Tubes	4 µg/mL BPDE-DNA (µL)	4 µg/mL Reduced DNA (µL)	BPDE-DNA Conc. (ng/mL)
1	10	390	100
2	200 of tube #1	200	50
3	200 of tube #2	200	25
4	200 of tube #3	200	12.5
5	200 of tube #4	200	6.25
6	200 of tube #5	200	3.13
7	200 of tube #6	200	1.56
8	0	200	0

Table 1. Preparation of BPDE-DNA Calibration Curve

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 calibration curve.
3. Add 50 µL of unknown DNA samples or BPDE-DNA calibrators 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 calibrator 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.

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 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.

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 Calibrator as an absorbance blank.

EXAMPLE OF RESULTS

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

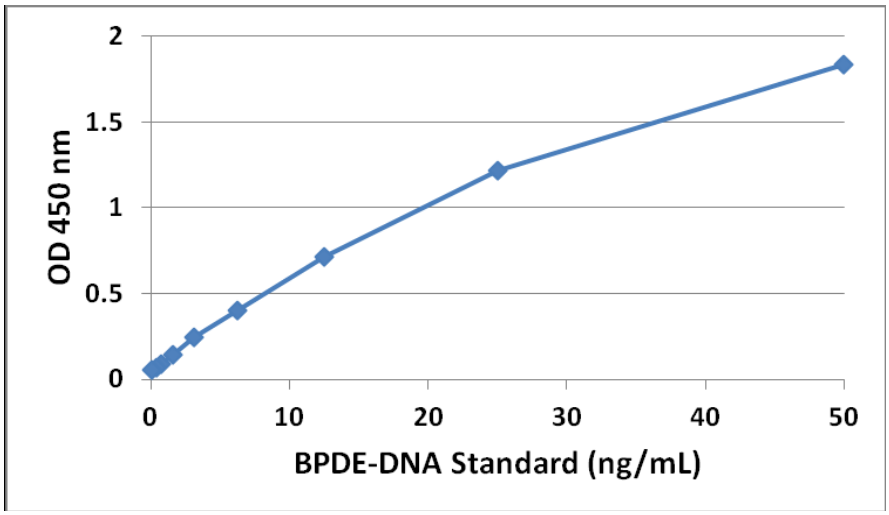

Figure 2: BPDE-DNA Calibration Curve

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