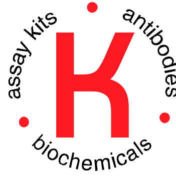

KAMIYA BIOMEDICAL COMPANY

BPDE Protein Adduct ELISA

For rapid detection of BPDE-protein adducts.

Cat. No. KT-904

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.

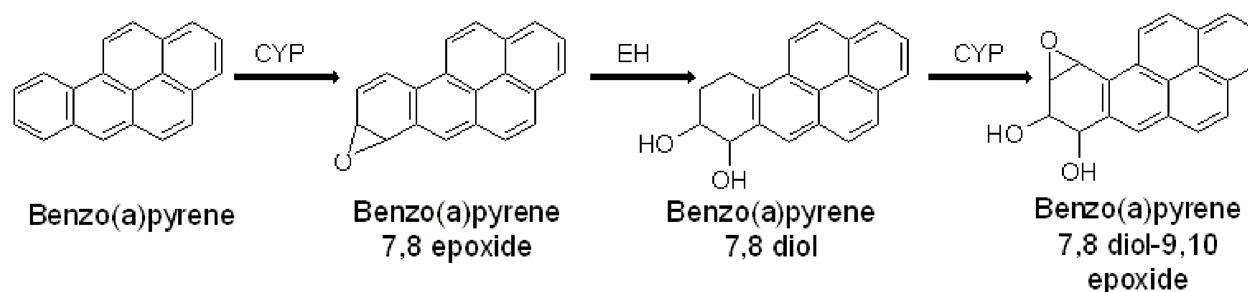

Figure 1: Benzo(a)pyrene catalyzed to various metabolites by Cytochrome P450 enzymes (CYP) and epoxide hydrolase (EH), resulting in the final carcinogen BPDE.

The BPDE Protein Adduct ELISA Kit is an enzyme immunoassay developed for rapid detection of BPDE-protein adducts. The quantity of BPDE adduct in protein samples is determined by relative comparison of a known BPDE-BSA calibration curve. Each kit provides sufficient reagents to perform up to 96 assays, including calibration curve and unknown protein samples.

ASSAY PRINCIPLE
BSA calibrators or protein samples (10 µg/mL) are adsorbed onto a 96-well plate for 2 hrs at 37ºC. The BPDE-protein adducts present in the sample or calibrator are probed with an Anti-BPDE-I Antibody, followed by an HRP Conjugated Secondary Antibody. The BPDE protein adduct content in an unknown sample is determined by comparing with a calibration curve that is prepared from predetermined BPDE-BSA calibrators.

COMPONENTS
1. <u>96-well Protein Binding Plate</u>: One strip well 96-well plate.
2. <u>Anti-BPDE Antibody (1,000X)</u>: One 20 µL vial of anti-BDPE-I antibody.
3. <u>Secondary Antibody, HRP Conjugate (1,000X)</u>: One 20 µL vial.
4. <u>Assay Diluent</u>: One 50 mL bottle.
5. <u>10X Wash Buffer</u>: One 100 mL bottle.
6. <u>Substrate Solution</u>: One 12 mL amber bottle.
7. <u>Stop Solution</u>: One 12 mL bottle.
8. <u>Reduced BSA Calibrator</u>: One 500 µL vial of 1 mg/mL reduced BSA in PBS.
9. <u>BPDE-BSA Calibrator</u>: One 20 µL vial of 1 mg/mL BPDE-BSA in 1X PBS.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Protein samples such as purified protein, plasma, serum, cell lysate
2. 1X PBS
3. 10 µL to 1,000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

STORAGE

Upon receipt, aliquot and store the Reduced BSA and BPDE-BSA 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 Concentrate 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.

CALIBRATOR PREPARATION

1. Freshly prepare 10 µg/mL of Reduced BSA by diluting the 1 mg/mL BSA calibrator in 1X PBS. Example: Add 50 µL to 4.95 mL of 1X PBS.
2. Freshly prepare 1 µg/mL of BPDE-BSA by diluting the 1 mg/mL BPDE-BSA calibrator in 10 µg/mL of Reduced BSA. Example: Add 2 µL to 2.0 mL of 10 µg/mL Reduced BSA.
3. Prepare a series of BPDE-BSA calibrators according to Table 1.

Calibrator Tubes	**1 µg/mL BPDE-BSA (µL)**	**10 µg/mL Reduced BSA (µL)**	**Final BPDE-BSA Conc. (ng/mL)**
1	1,000	0	1,000
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 BPDE-BSA Calibration Curve

SAMPLE PREPARATION

1. Perform a protein assay such as Bradford or BCA on all samples to determine the protein concentration.
Note: Cell and tissue lysates should not be prepared in lysis buffer containing Triton X-100, NP-40, or Igepal CA-630 because these detergents interfere with protein coating of the plate unless the detergent concentration in the 10 µg/mL protein samples is no more than 0.001%. We recommend lysis by homogenization or sonication.
2. Dilute each protein sample to 10 µg/mL in 1X PBS prior to use in the assay.

ASSAY PROCEDURE

1. Prepare unknown samples according to the Preparation of Samples section above. Each 10 µg/mL protein sample and BSA Calibrator should be assayed in duplicate or triplicate.

2. Add 100 µL of the 10 µg/mL protein samples or Reduced/BPDE-BSA calibrators to the 96-well Protein Binding Plate. Incubate at 37ºC for at least 2 hours or 4ºC overnight.

3. Wash wells 2 times with 250 µL 1X PBS per well. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess wash solution.

4. Add 200 µL of Assay Diluent per well and incubate for 1-2 hours at room temperature on an orbital shaker.

5. Wash 3 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.

6. 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. Wash the strip wells 3 times according to step 5 above.

7. 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 5 above.

8. 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.

9. Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time).

10. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length. Use the Reduced BSA Calibrator as an absorbance blank.

EXAMPLE OF RESULTS

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

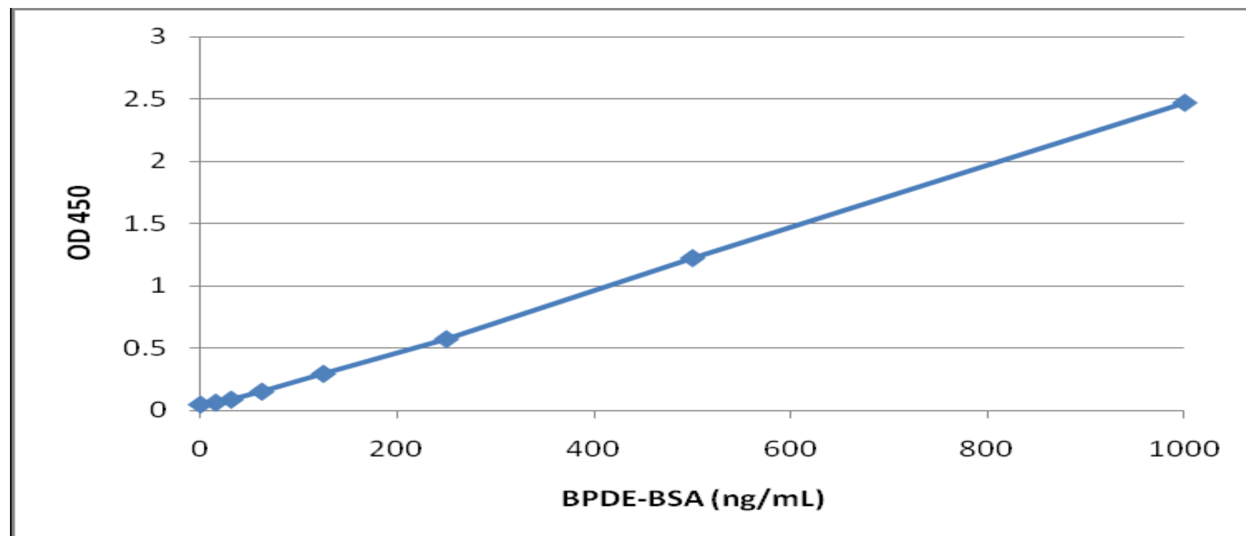

Figure 2: BPDE-BSA Calibration Curve

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