BPDE Protein Adduct ELISA Kit

Enzyme Immunoassay for the quantification of BPDE Protein Adduct in purified protein, plasma, serum, cell lysate.

Catalog number: ARG81226

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INTRODUCTION

Benzo[a]pyrene is a polycyclic aromatic hydrocarbon and the result of incomplete combustion of organic matter at temperatures between 300 °C (572 °F) and 600 °C (1,112 °F). The ubiquitous compound can be found in coal tar, tobacco smoke and many foods, especially grilled meats. The substance with the formula C20H12 is one of the benzopyrenes, formed by a benzene ring fused to pyrene. Its diol epoxide metabolites (more commonly known as BPDE) react and bind to DNA, resulting in mutations and eventually cancer. It is listed as a Group 1 carcinogen by the IARC. In the 18th century a scrotal cancer of chimney sweepers, the chimney sweeps' carcinoma, was already known to be connected to soot. [Provide by Wikipedia: Benzo(a)pyrene]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. Standards or protein samples (10 µg/mL) are pipetted into the wells for 2 hours at 37°C or overnight at 4°C. An antibody-conjugate specific for BPDE is added to each well and incubate. After washing away any unbound antibody, an HRP conjugate antibody added to the wells. After washing away any unbound substances, the TMB substrate is added to the wells and color develops in proportion to the amount of BPDE protein adduct bound with the antibody-conjugate. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450nm. The concentration of BPDE protein adduct in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, aliquot and store the BPDE-BSA Standard at -20°C to avoid multiple freeze/thaw cycles. Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Protein Binding microplate	8 X 12 strips	4°C
Standards (1 mg/ml BPDE-BSA in PBS)	20 µL	–20°C.
Reduced Standards (1 mg/ml reduced BSA in PBS)	500 µL	4°C
Diluent Buffer	50 ml (ready to use)	4°C
Antibody Conjugate (Anti-BPDE-I antibody)	20 µL	4°C
HRP Conjugate Antibody.	20 µL	4°C
10X Wash Buffer	100 ml	4°C
TMB substrate	12 ml (ready to use)	4°C (protect from light)
STOP solution	12 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- 1X PBS
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- The Stock Standard should be aliquoted into smaller portions before use to ensure product integrity and store the aliquoted Stock Standard at -20°C. Avoid repeated freeze-thaw cycles
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, do NOT

use it.

- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Use a new adhesive plate cover for each incubation step.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g and 4°C for 10 minutes. Collect the plasma layer and store on ice.

Cells and tissue lysate: Homogenize 50-200 mg of the cell and tissue pellet in 0.5-2 mL of ice cold PBS using a mortar and pestle or by dounce homogenization. Incubate the homogenate at 4°C for 20 minutes. Transfer the homogenate to a centrifuge tube and centrifuge at 12000 x g for 20 minutes. Recover the supernatant and transfer to a fresh tube. Store resuspended sample at -20°C or colder until ready to test by ELISA.

Sample protein quantification:

1. Perform a protein assay such as Bradford or BCA on all samples to determine the protein concentration.
2. Dilute each protein sample to 10 µg/mL in 1X PBS prior to use in the assay.

Note:

1. Mouse and rat plasma and serum are not compatible with this assay.
2. Cell and tissue lysates should not be prepared in lysis buffer containing Triton X-100, NP-40, or IGEPAL CA-630.
3. Do not use haemolytic, icteric or lipaemic specimens.
4. Avoid disturbing the white buffy layer when collection serum/plasma sample.
5. Aliquot samples for testing and store at -80°C. Avoid repeated freeze-thaw cycles. Perform dilutions in Diluent buffer as necessary.
6. Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. (E.g., add 50 ml of 10X Wash buffer into 450 ml of distilled water to a final volume of 500 ml) The 1X Washing Buffer is stable for up to 4 weeks at 2-8°C.
- Antibody conjugate and HRP conjugate antibody: Immediately before use dilute the Antibody conjugate (Anti-BPDE-I antibody 1:1000 and the HRP Conjugate antibody 1:1000 with Diluent buffer. Do not store diluted solutions.
- Standards (BPDE-BSA):
    1. Freshly prepare 10 µg/mL of Reduced BSA by diluting the 1 mg/mL BSA standard 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 standard 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 dilution of BPDE-BSA standards with Reduced standards. The Reduced standards serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted with Reduced standards as according to the suggested concentration table below:

Standard tubes	Final BPDE-BSA (ng/mL)	Reduced Standards (µL)	Standards (µL)
S1	1000	0	1000 of 1 µg/ml stock standard
S2	500	500	500 of S1
S3	250	500	500 of S2
S4	125	500	500 of S3
S5	62.5	500	500 of S4
S6	31.3	500	500 of S5
S7	15.6	500	500 of S6
S0	0	500	0

Note: Dilutions for the standard must be made and applied to the plate immediately. S0 serves as background.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Prepare samples according to the SAMPLE COLLECTION & STORAGE INFORMATION above. Each 10 µg/mL protein sample and BSA Standard should be assayed in duplicate or triplicate.

1. Add **100 µL** of **sample or standard** to the Protein Binding microplate.
2. Incubate at **37°C** for at least **2 hours** or **4°C overnight** on a microplate shaker.
3. Aspirate each well and wash, repeating the process 1 times for a total 2 washes. Wash by filling each well with **1× Wash Buffer (250 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **200 µL** of Diluent Buffer to each well. Incubate at **RT** for **1-2 hours** on a microplate shaker.
5. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with **1× Wash Buffer (250 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add **100 µL** of the diluted **Antibody Conjugate** to each well. Incubate at **RT** for **1 hour** on a microplate shaker.
7. Aspirate each well and **wash as step 5**.
8. Add **100 µL** of the diluted **HRP Conjugate** to each well. Incubate at **RT** for **1**

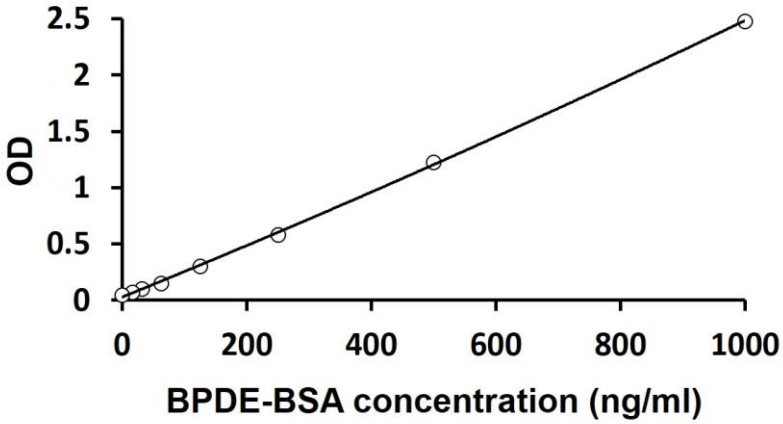
hour on a microplate shaker.

9. Aspirate each well and wash as step 5.
10. Warm Substrate Solution to room temperature. Add 100 µl of TMB Substrate to each well, including the blank wells. Incubate for 2-30 minutes at room temperature in the dark.

    Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
11. Add 100 µl of Stop Solution to each well, including the blank wells. The color of the solution should change from blue to yellow.
12. Read the OD with a microplate reader at 450nm immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance within 30 minutes after adding the stop solution.

EXAMPLE OF TYPICAL STANDARD CURVE

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



CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for the detail. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

QUALITY ASSURANCE

Intra-assay and Inter-assay precision

The CV value of intra-assay and inter-assay precision was ≤ 10%.

Sensitivity

10 µg/ml