TREVIGEN® Instructions

For Research Use Only. Not For Use In Diagnostic Procedures

HT BPDE ELISA Kit

96 Tests

Cat# 4360-096-K

High throughput ELISA to quantify BPDE adducts in plasma, serum, and cell culture supernatant samples

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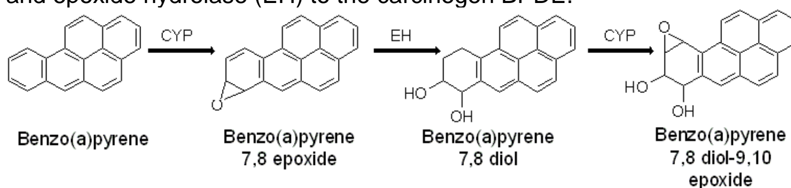
I. Introduction

Benzo(a)pyrene (BP), belonging to polycyclic aromatic hydrocarbons (PAHs), is a widespread environmental pollutant found in cigarette smoke, charred food, exhaust from internal combustion engines and coal-burning factories [1]. A highly reactive diol epoxide derivative, BPDE, is the major electrophilic, mutagenic, and carcinogenic metabolite involved in covalent binding to DNA and proteins (Figure 1) [2]. Bound BPDE adducts serve as a biomarker for exposure as well as the body's metabolic response to this environmental mutagen associated with the increased risk of lung and breast cancers [3-6].

Figure 1: Enzymatic conversion of Benzo(a)pyrene by Cytochrome P450 (CYP) and epoxide hydrolase (EH) to the carcinogen BPDE.



BPDE is also a frequently used biomarker of oxidative DNA damage and oxidative stress. To address this need Trevigen offers a validated HT BPDE competitive ELISA kit, a fast and sensitive immunoassay for the detection and quantitation of BPDE adducts in plasma, serum and cell culture supernatant samples. The assay employs a pre-coated BPDE-HSA monoclonal antibody, a labeled Biotinylated BPDE-HSA, a Strep-HRP conjugate, and chemiluminescent detection substrate to construct a high throughput assay flexible for your experimental design. Important features of the assay include: 1) chemiluminescent, non-radioactive format; 2) high throughput 96 strip wells; 3) dynamic range from 6.25 nM to 400 nM BPDE-HSA; and, 4) sensitivity at 5 nM BPDE-HSA.

II. Precautions and Limitations

1. For Research Use Only. Not for use in diagnostic procedures.
2. The physical, chemical, and toxicological properties of the chemicals and reagents contained in the HT BPDE ELISA Kit may not yet have been fully investigated. Therefore, Trevigen recommends the use of gloves, lab coats, and eye protection while using any of these chemical reagents. Trevigen assumes no liability for damage resulting from handling or contact with these products. MSDS are available on request.
3. The components in each kit lot number have been validated in this specific combination only; please do not mix them with components from other kits.

III. Materials Supplied

Catalog Number	Component	Amount Provided	Storage Temperature
4360-096-01	BPDE-HSA Standard, 10 µM	40 µl	-20°C
4360-096-02	Assay Diluent	50 ml	4°C
4360-096-03	Biotinylated BPDE-HSA	20 µl	-20°C
4360-096-P	Pre-coated 96-stripwell plate	1 plate	4°C
4800-30-06	Strep-HRP	30 µl	4°C
4675-096-01	PeroxyGlow™ A	6 ml	4°C
4675-096-02	PeroxyGlow™ B	6 ml	4°C

IV. Materials/Equipment Required But Not Supplied

Reagents/Disposables:

1. Biological specimens to be tested
2. 1XPBS containing 0.1% Tween 20 (PBST)
3. Pipette and tips
4. Microcentrifuge tubes and conical tubes

Equipment:

1. Pipette-aid, pipettor and multichannel pipettor
2. Wash bottle or microstrip wells plate washer (optional)
3. 96-well chemiluminescent plate reader or luminometer
4. Vortexer and microcentrifuge
5. Heat block
6. Incubator set at 25 ºC
7. -20 ºC and 4 ºC storage

V. Reagent Preparation

1. PBS + 0.1% Tween 20 Wash Solution (PBST)

Prepare 500 ml of 1X PBST containing 1X PBS and 0.1% Tween 20 in a wash bottle for washing strip wells.

2. BPDE-HSA Standard

The kit contains 40 µl of BPDE-HSA standard (Cat# 4360-096-01) at a concentration of 10 µM. Centrifuge the standard vial before opening cap. Aliquot and avoid repeated freeze/thaw cycles. Serially dilute the standard with Assay Diluent (Cat# 4360-096-02) just before use. The volume of each dilution should be 100 µl or greater. The recommended final concentrations are 400, 200, 100, 50, 25, 12.5, and 6.25 nM. The standard curve requires 25 µl/well of each dilution performed in triplicate. Table 1 describes a serial dilution protocol for BPDE-HSA standards. **Diluted BPDE-HSA standard should be used immediately and any remainder discarded.**

Table 1: Dilution of BPDE-HSA Standard

BPDE-HSA Concen.	400 nM	200 nM	100 nM	50 nM	25 nM	12.5 nM	6.25 nM
BPDE-HSA Standard 10 µM →	8 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl
Assay Diluent	192 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl

3. Biotinylated BPDE-HSA

Centrifuge the Biotinylated BPDE-HSA vial before opening cap. Aliquot and avoid repeated freeze/thaw cycles. Just before use, dilute Biotinylated BPDE-HSA (Cat# 4360-096-03) 250-fold with Assay Diluent (Cat# 4360-096-02). A total of 25 µl/well of diluted Biotinylated BPDE-HSA is required in the assay. For 96 wells, dilute 12 µl of Biotinylated BPDE-HSA into 3 ml of Assay Diluent and add 25 µl/well with a multichannel pipettor.

4. Strep-HRP Conjugate

Just before use, dilute Strep-HRP Conjugate (Cat# 48000-30-06) 500-fold with Assay Diluent (Cat# 4360-096-02). A total of 50 µl/well of diluted Strep-HRP Conjugate is required in the assay. For 96 wells, dilute 12 µl of Strep-HRP Conjugate into 6 ml of Assay Diluent and add 50 µl/well with a multichannel pipettor.

5. PARP PeroxyGlow™ A and B Chemiluminescent Substrates

Allow PARP PeroxyGlow™ A and B to come to room temperature. Just before use, mix equal volumes of PARP PeroxyGlow™ A and B together. A total of 100 µl is required per well. PARP PeroxyGlow™ A and B are Horseradish Peroxidase (HRP) substrates generating light that can be quantified in a suitable chemiluminescent plate reader. For example, for a whole plate, mix 6 ml of PARP PeroxyGlow™ A with 6 ml of PARP PeroxyGlow™ B together and add 100 µl/well with a multichannel pipettor.

VI. Preparation of Sample

i. Plasma samples:

1. Withdraw blood per standard procedures using Sodium Heparin or EDTA as anticoagulant.
2. Collect plasma by centrifugation at room temperature in a horizontal rotor (swinging bucket) in a proper adaptor for 15 minutes at 1,500 x g within 30 minutes of blood collection.
3. Carefully transfer the plasma and centrifuge for another 15 minutes at 2,500 x g at room temperature.
4. Carefully transfer the supernatant and assay immediately or store at ≤ -20 ^{o}C in aliquots for later use. Avoid repeated freeze-thaw cycles.

ii. Serum Samples:

1. Collect whole blood using established methods.
2. Allow samples to clot at room temperature for 30 minutes.
3. Centrifuge at 2,700 × g for 10 minutes, taking precautions to avoid hemolysis.
4. Carefully remove the serum and assay immediately or store at ≤ -20 ºC in aliquots for later use. Avoid repeated freeze-thaw cycles.

iii. Cell culture supernatants:

Remove particulates and clarify cell culture supernatant or fluid by centrifugation 15 minutes at 1,500 x g and assay immediately or aliquot and store samples at ≤ -20 ºC. Avoid repeated freeze-thaw cycles.

VII. Assay Protocol

1. Remove strip wells from foil pouch and bring to room temperature. See Section XII Appendix for sample plate layout.

   Note: If less than 96 pre-coated wells are needed, remove excess strips from frame, return to foil pouch and store at 4 °C with desiccant. (Data performance will be compromised if desiccant color changes from blue to pink).

2. Prepare BPDE-HSA Standard (Section V item 2) and samples (Section VI). 100 µl final volumes of the standards are recommended to assay in triplicate. Clarified samples can be directly applied for testing.

   NOTES:
   If samples generate values greater than the 400 nM standard, dilute the samples in Assay Diluent until the value fall in the dynamic range.

3. Add 25 µl of BPDE-HSA standards and 25 µl clarified samples to appropriate wells. Add 25 µl of Assay Diluent to 0 nm BPDE-HSA and blank wells (background control). See Section XII Appendix Table 2 for sample plate layout.

4. Add 25 µl of Biotinylated BPDE-HSA solution (Section V item 3) to all wells except blank wells. Add 25 µl of Assay Diluent to blank wells instead. Mix thoroughly without causing air bubbles. Cover wells with film sealer and incubate at 25 °C for 1 hour.

5. Gently remove film sealer and assay contents. Wash wells 4 times with PBST (300 µl/well). Ensure liquid is removed by tapping plate onto paper towels between each wash.

6. Don't let the plate wells to dry out and **Immediately** add 50 µl of diluted Strep-HRP conjugate (Section V item 4) to all wells. Cover wells with film sealer and incubate at 25°C for 1 hour. Place the PeroxyGlow™ A and B reagents at 25 ºC to pre-warm.

7. Gently remove the film sealer and assay contents. Wash wells 4 times with PBST (300 µl/well). Ensure liquid is removed by tapping plate onto paper towels between each wash.

8. Just before use, mix equal volumes of PeroxyGlow™ A and B together and add 100 µl per well. **Immediately** take chemiluminescent readings.

VIII. Data Interpretation

Determination of BPDE adduct concentrations on the Web

1) Use Calculations Worksheet (MS Excel is required) provided on the Trevigen website product page (4360-096-K).
2) Follow instruction steps on Calculations Worksheet.

Alternative Method to Determine BPDE adduct concentrations

1) Calculate the average RLU measurement for each BPDE-HSA Standard, sample and blank.
2) Subtract the blank average from BPDE-HSA Standard and sample averages to determine the relative RLU.
3) Plot the log of BPDE-HSA Standard concentrations (nM) on the X-axis versus the relative RLU on the Y-axis.
4) The standard curve is a 2nd order polynomial function represented by the equation: $y = a + bx + cx^2$, where y is the relative RLU, x is the log of BPDE-HSA concentration in nM and a, b and c are coefficients. Calculate the sample BPDE adduct concentrations using the polynomial equation or interpolation from the standard curve.
5) Multiply by the dilution factor for the final sample BPDE adduct concentrations. For example, if the sample was diluted 1:2, the value generated from the polynomial equation or the standard curve must be multiplied by 2 to calculate the final sample BPDE adduct concentrations.

Example data

The following figures are examples of BPDE ELISA Kit results. The data below is for reference only and should not be used to interpret actual results.

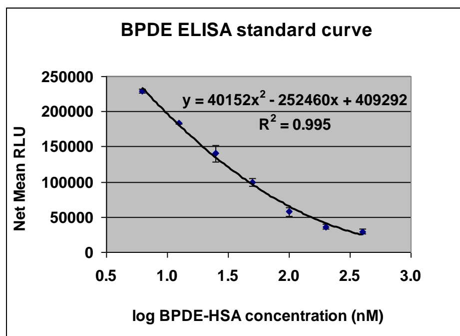


Figure 1. Typical BPDE-HSA Standard Curve. BPDE-HSA Standards were prepared in Section V and assayed per the protocol in Section VII.

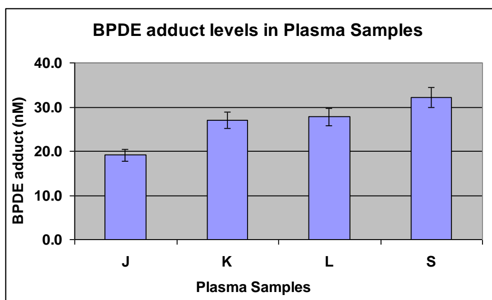


Figure 2. BPDE adduct levels in plasma samples from healthy donors. Validation of assay reproducibility was determined by obtaining biofluid samples from four healthy donors. Sample extracts were prepared as described in Section VI.

IX. Performance Characteristics

Intra-Assay Precision: The intra-assay precision (within-run precision): three samples of known concentration were tested nine times in a single run. The overall intra-assay coefficient of variation was calculated to be <15%.

Inter-Assay Precision: The inter-assay precision (between-run precision): three samples of known concentration were tested nine times in three separate runs. The overall inter-assay coefficient of variation was calculated to be <20%.

Sensitivity of the ELISA: The LLD (lower limit of detection) of BPDE-HSA was calculated to be 5 nM.

Spike and Recovery: Test samples were spiked with three different levels of BPDE-HSA and analyzed for recovery before and after spiking. The calculated overall mean is between 80%-120%.

Dilution Linearity: Test samples were serially diluted in the Assay Diluent and subsequently measured by the assay. Dilution recovery is assessed by comparing observed vs. expected values based on undiluted samples. The calculated overall mean is between 80%-120%.

X. References

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5. S.S. Hecht, Tobacco smoke carcinogens and lung cancer, Journal of the National Cancer Institute 91 (14) (1999) 1194–1210.
6. J.P. Butler, G.B. Post, P.J. Lioy, J.M. Waldman, A. Greenberg, Assessment of carcinogenic risk from personal exposure to benzo(a) pyrene in the Total Human Environmental Exposure Study (THEES), Air and Waste 43 (7) (1993) 970–977.

XI. Related Products Available From Trevigen

Kits:

Catalog #	Description	Size
4380-096-K	HT 8-oxo-dG ELISA kit II	96 tests
4380-192-K	HT 8-oxo-dG ELISA kit II	192 tests
4520-096-K	PARP in vivo Pharmacodynamic Assay II	96 tests
4700-096-K	Tankyrase 1 (PARP5A) Colorimetric Activity Assay	96 tests
4700-192-K	Tankyrase 1 (PARP5A) Colorimetric Activity Assay	192 tests
4701-096-K	Tankyrase 1 (PARP5A) Chemiluminescent Activity Assay	96 tests
4701-192-K	Tankyrase 1 (PARP5A) Chemiluminescent Activity Assay	192 tests
4691-096-K	HT Homogeneous PARG Inhibition Assay	96 tests
4676-096-K	Universal Chemiluminescent PARP Assay Kit/w Histone Coated Strip Wells	96 tests
4677-096-K	Universal Colorimetric PARP Assay Kit/w Histone Coated Strip Wells	96 tests
4684-096-K	HT Colorimetric PARP Apoptosis Assay	96 tests
4685-096-K	HT Chemiluminescent PARP Apoptosis Assay	96 tests
4690-096-K	HT F Homogeneous PARP Inhibition Assay Kit	96 tests
4682-096-K	HT Chemiluminescent PARG Assay Kit	96 tests
4683-096-K	HT Colorimetric PARG Assay Kit	96 tests

Inhibitors and Antibodies:

Catalog #	Description	Size
4667-50-03	3-Aminobenzamide (200 mM)	100 µl
4335-AMC-050	PAR Monoclonal Antibody Affinity Purified	50 µl
4335-MC-100	Anti-PAR Monoclonal Antibody	100 µl
4336-APC-050	PAR Polyclonal Antibody Affinity Purified	50 µl
4336-BPC-100	Anti-PAR Polyclonal Antibody (rabbit)	50 µl
4338-MC-50	Anti-PARP Monoclonal Antibody (clone C2-10)	50 µg
2305-PC-100	Anti-Cleaved Caspase 3 Polyclonal	100 µl

Poly(ADP)-ribose:

Catalog #	Description	Size
4336-100-01	Poly(ADP) Ribose (PAR) Polymer	100 µl

XII. Appendix

Table 2: Sample plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	400 nM	400 nM	400 nM	Sample 1	Sample 1	Sample 1						
B	200 nM	200 nM	200 nM	Sample 2	Sample 2	Sample 2						
C	100 nM	100 nM	100 nM	Sample 3	Sample 3	Sample 3						
D	50 nM	50 nM	50 nM	Sample 4	Sample 4	Sample 4						
E	25 nM	25 nM	25 nM	Sample 5	Sample 5	Sample 5						
F	12.5 nM	12.5 nM	12.5 nM	Sample 6	Sample 6	Sample 6						
G	6.25 nM	6.25 nM	6.25 nM	Sample 7	Sample 7	Sample 7						
H	0 nM	0 nM	0 nM	Blank	Blank	Blank						

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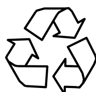

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