TREVIGEN® **Instructions**

For Research Use Only. Not For Use In Diagnostic Procedures

HT BPDE Sandwich ELISA Kit

96 Tests

Cat# 4362-096-K

High throughput ELISA to quantify BPDE-HSA in human plasma and serum samples

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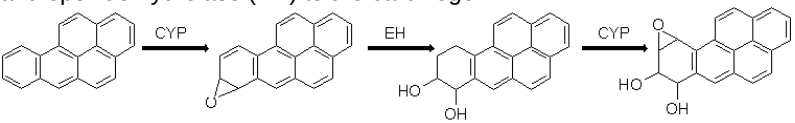
Table of Contents

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]. Human BPDE-HSA adducts have inherent advantages over DNA adducts as measures of exposure for epidemiologic studies [7].

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 stress. To address this need Trevigen offers a validated HT BPDE Sandwich ELISA kit for the detection and quantitation of BPDE-HSA in human plasma and serum samples. The assay employs a pre-coated anti-BPDE monoclonal capture antibody, a biotinylated anti-human HSA polyclonal detecting antibody, and a chemiluminescent detection substrate, generating a highly sensitive and 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 0.63 nM to 40 nM BPDE-HSA; and, 4) high sensitivity (LLD of 0.5 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 Sandwich 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 is available on request.
3. The components in each kit lot number have been quality assured and validated in this specific combination only; please do not mix them with components from other kit lot numbers.

III. Materials Supplied

Catalog Number	Component	Amount Provided	Storage Temperature
4362-096-01	BPDE-HSA Standard, 4 µM	20 µl	-20°C
4362-096-02	Sample Buffer	15 ml	4°C
4362-096-03	Biotinylated anti-HSA polyclonal antibody	20 µl	-20°C
4362-096-04	Assay Buffer	20 ml	4°C
4362-096-P	Pre-coated 96-stripwell plate	1 plate	4°C
4675-096-01	PeroxyGlow™ A	6 ml	4°C
4675-096-02	PeroxyGlow™ B	6 ml	4°C
4800-30-06	Strep-HRP conjugate	20 µl	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 plate reader with 450 nm filter
4. Vortex 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 Standards

The kit contains 20 µl of BPDE-HSA standard (Cat# 4362-096-01) at a concentration of 4 µM. Centrifuge the standard vial before opening cap. Aliquot and avoid repeated freeze/thaw cycles.

Serially dilute the standard with Sample Buffer (Cat# 4362-096-02) just before use. The volume of each dilution should be 200 µl or greater. The recommended standard concentrations are 40 nM, 20 nM, 10 nM, 5.0 nM, 2.5 nM, 1.25 nM and 0.63 nM. The standard curve requires 50 µl/well of each BPDE-HSA standard in triplicate wells. 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 Concen. (nM)	40 nM	20 nM	10 nM	5.0 nM	2.5 nM	1.25 nM	0.63 nM
BPDE Standard 4 µM	4 µl	200 µl	200 µl	200 µl	200 µl	200 µl	200 µl
Sample Buffer	396 µl	200 µl	200 µl	200 µl	200 µl	200 µl	200 µl

3. Biotinylated Anti-HSA Polyclonal Antibody

Just before use, dilute Biotinylated anti-HSA polyclonal rabbit antibody (Cat# 4362-096-03) 500-fold with Assay Buffer (Cat# 4362-096-04). A total of 50 µl/well of diluted Biotinylated anti-HSA polyclonal antibody is required in the assay. For 96 wells, dilute 12 µl of Biotinylated anit-HSA polyclonal antibody into 6 ml of Assay Buffer and aliquot 50 µl/well with a multichannel pipettor.

4. Strep-HRP Conjugate

Just before use, dilute Strep-HRP conjugate (Cat# 4800-30-06) 500-fold with Assay Buffer (Cat# 4362-096-04). 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 Buffer and aliquot 50 µl/well with a multichannel pipettor.

5. PeroxyGlow™ A and B Chemiluminescent Substrates

Equilibrate PeroxyGlow™ A and B to room temperature. Just before use, mix equal volumes of PeroxyGlow™ A and B together. A total of 100 µl is required per well. Light signal generated by these Horseradish Peroxidase (HRP) substrates are quantified using a chemiluminescent plate reader. For 96 wells, mix 6 ml PeroxyGlow™ A and 6 ml PeroxyGlow™ B and aliquot 100 µl/well with a multichannel pipettor.

VI. Preparation of Sample

i. Plasma samples:

1. Withdraw blood according to 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 ºC 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 transfer the serum and assay immediately or store at ≤ -20 ºC in aliquots for later use. Avoid repeated freeze-thaw cycles.

VII. Assay Protocol

1. Remove strip wells from foil pouch and bring to room temperature. See Section XI 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 standards (Section V item 2) and samples (Section VI). 200 µl of each BPDE-HSA standard is recommended to assay in triplicate. In order to minimize non-specific HSA binding from samples, a loading volume of 25 µl/well is recommended.

   NOTE: An initial 1:2 dilution of samples in Sample Buffer is recommended. If samples generate values greater than the 40 nM standard, make further dilutions with Sample Buffer until the values fall within the dynamic range of the assay.

3. Carefully add 50 µl of each BPDE-HSA standard and 25 µl of each diluted sample to the bottom center of appropriate wells. Gently shake the plate so solutions cover well bottoms. Cover wells with film sealer and incubate at 25 °C for 2 hour.

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

5. Aliquot 50 µl of diluted Biotinylated anti-HSA polyclonal antibody (Section V item 3) to all wells. Cover wells with film sealer and incubate at 25 °C for 1 hour.

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

7. Aliquot 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 PeroxyGlow™ A and B at 25 ºC to equilibrate.

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

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

VIII. Data Interpretation

Determination of BPDE-HSA concentrations on the Web

Use Calculations Worksheet provided on Trevigen website: http://www.trevigen.com/BPDE-ELISA-II.php. (MS Excel is required).

Alternative Method to Determine BPDE-HSA concentrations

1) Calculate the average light unit (LU) measurement for each BPDE-HSA Standard, sample and blank.
2) Subtract the blank average LU from BPDE-HSA Standard and sample averages to determine the relative RLU.
3) Plot the BPDE-HSA Standard concentrations (nM) on the X-axis versus their 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 BPDE-HSA standard concentration in nM and a, b and c are coefficients. Calculate the sample BPDE-HSA concentrations using the polynomial equation or interpolate from the standard curve.
5) To determine final BPDE-HSA sample concentrations, multiply by the sample dilution. 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 determine the final sample BPDE-HSA concentration.

Example data

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

Figure 2. Typical BPDE-HSA Standard Curve. BPDE-HSA Standards were prepared in Section V and assayed according to the protocol in Section VII.

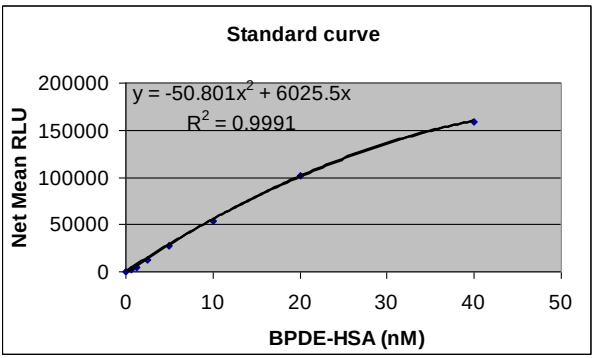


Table 2. BPDE-HSA levels in plasma samples from healthy donors. Validation of assay reproducibility was determined by obtaining biofluid samples from four healthy donors. Sample extracts were prepared as described in Section VI. General BPDE-HSA concentrations reported for unexposed controls are between 7.5–229 fmol BPDE-HSA / mg HSA [7].

Sample ID	PAH exposure	BPDE–HSA (nM)	BPDE–HSA (fmol / mg of HSA)
A	Low	5.26 ± 0.19	105.2 ± 3.8
B	Low	2.18 ± 0.07	43.6 ± 1.4
C	Low	4.12 ± 0.43	82.4 ± 8.6

IX. 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.
7. M.K. Chung, J. Riby, H. Li, A.T. Iavarone, E.R. Williams, Y. Zheng, S.M. Rappaport, A sandwich enzyme-linked immunosorbent assay for adducts of polycyclic aromatic hydrocarbons with human serum albumin, Analytical Biochemistry 400 (2010) 123–129.

X. Related Products Available From Trevigen

Kits:

Catalog #	Description	Size
4360-096-K	HT BPDE ELISA Kit	96 tests
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

Antibodies:

Catalog #	Description	Size
4422-AMC-100	Anti-XPF Monoclonal Antibody (1E4)	100 µl
4424-AMC-100	Anti-ERCC1 Monoclonal Antibody (3G7)	100 µl
4335-AMC-50	PAR Monoclonal Antibody Affinity Purified	50 µl
4335-MC-100	Anti-PAR Monoclonal Antibody	100 µl
4336-APC-50	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

XI. Appendix

Table 2: Sample plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	40 nM	40 nM	40 nM	Sample 1	Sample 1	Sample 1						
B	20 nM	20 nM	20 nM	Sample 2	Sample 2	Sample 2						
C	10 nM	10 nM	10 nM	Sample 3	Sample 3	Sample 3						
D	5 nM	5 nM	5 nM	Sample 4	Sample 4	Sample 4						
E	2.5 nM	2.5 nM	2.5 nM	Sample 5	Sample 5	Sample 5						
F	1.25 nM	1.25 nM	1.25 nM	Sample 6	Sample 6	Sample 6						
G	0.63 nM	0.63 nM	0.63 nM	Sample 7	Sample 7	Sample 7						
H	0 nM (Blank)	0 nM (Blank)	0 nM (Blank)	Sample 8	Sample 8	Sample 8						

The product accompanying this document is intended for research use only and is not intended for diagnostic purposes or for use in humans.

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