

**NOTE: Revision to
Format and Assay Protocol**

Product Manual

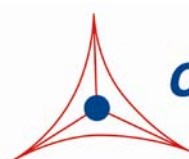
OxiSelect™ BPDE DNA Adduct ELISA Kit

Catalog Number

STA- 357

96 assays

**FOR RESEARCH USE ONLY
Not for use in diagnostic procedures**



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

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.

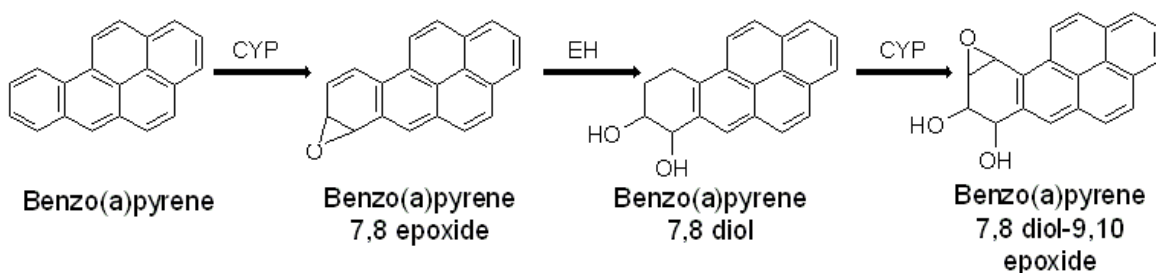


Figure 1: Benzo(a)pyrene catalyzed to various metabolites by Cytochrome P450 enzymes (CYP) and epoxide hydrolase (EH), resulting in the final carcinogen BPDE.

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

Assay Principle

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

Related Products

1. STA-301: OxiSelect™ BPDE Protein Adduct ELISA Kit
2. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
3. STA-321: OxiSelect™ DNA Double-Strand Break Assay
4. STA-322: OxiSelect™ UV-Induced DNA Damage ELISA Kit (CPD Quantitation)

5. STA-323: OxiSelect™ UV-Induced DNA Damage ELISA Kit (6-4PP Quantitation)
6. STA-324: OxiSelect™ Oxidative DNA Damage Quantitation Kit (AP Sites)
7. STA-325: OxiSelect™ Oxidative RNA Damage ELISA Kit (8-OHG Quantitation)
8. STA-350: OxiSelect™ Comet Assay Kit (3-Well)
9. STA-355: OxiSelect™ 96-Well Comet Assay Kit

Kit Components

Box 1 (shipped at room temperature)

1. DNA High-Binding Plate (Part No. 232404): One 96-well strip plate.
2. DNA Binding Solution (Part No. 232405): One 6 mL bottle.
3. Anti-BPDE Antibody (1000X) (Part No. 235601): One 20 µL vial of anti-BPDE-I antibody.
4. Secondary Antibody, HRP Conjugate (1000X) (Part No. 10902): One 50 µL vial.
5. Assay Diluent (Part No. 310804): One 50 mL bottle.
6. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
7. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
8. Stop Solution (Part. No. 310808): One 12 mL bottle.
9. Reduced DNA Standard (Part No. 235602): One 200 µL vial of 0.2 mg/mL reduced DNA in TE Buffer.

Box 2 (shipped on blue ice packs)

1. BPDE-DNA Standard (Part No. 235603): One 30 µL vial of 0.1 mg/mL BPDE-DNA in TE Buffer.

Materials 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 1000 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 Standards at -20°C to avoid multiple freeze/thaw cycles. Store all other kit components at 4°C.

Preparation of Reagents

- 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 1:1000 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

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 standards according to Table 1.

Standard 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 Standard Curve

Assay Protocol

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 standard curve.
3. Add 50 µL of unknown DNA samples or BPDE-DNA standards 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 standard 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 μ L 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 μ L 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 μ L 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 μ L 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 μ L 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 Standard 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.

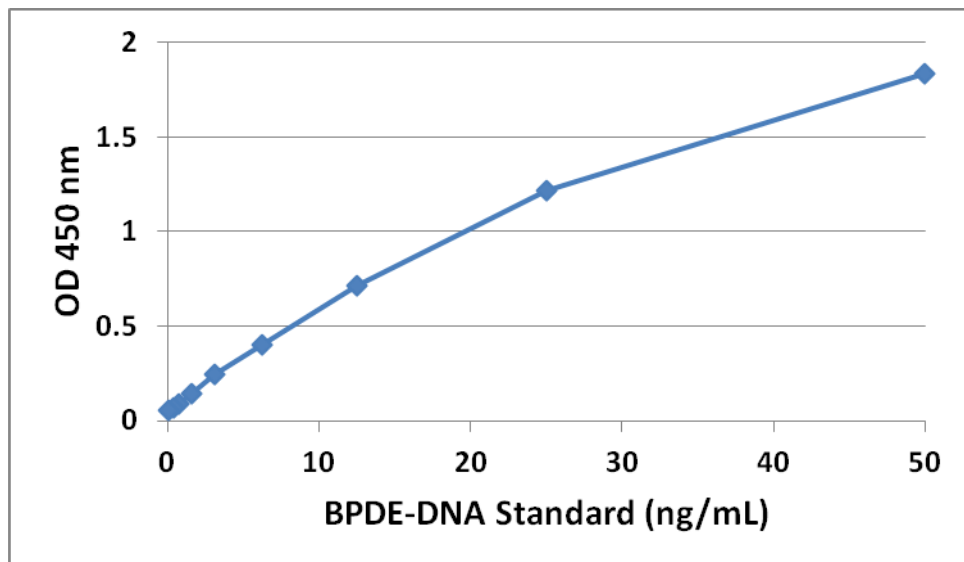


Figure 2: BPDE-DNA Standard Curve

References

1. Izzotti A, Rossi GA, Bagnasco M, De Flora S. (1991) *Carcinogenesis* **12**:1281-1285.
2. Denissenko MF, Pao A, Tang M, Pfeifer GP (1996) *Science* **274**:430-432.
3. Lee, B.M. and Santella, R.M. (1988) *Carcinogenesis*, **9**: 1773-1777.
4. Tang D, Phillips DH, Stampfer M, Mooney LA, Hsu Y, Cho S, Tsai WY, Ma J, Cole KJ, She MN, Perera FP. (2001) *Cancer Research* **61**:6708–6712.
5. Mumford JL, Williams K, Wilcosky TC, Everson RB, Young TL, Santella RM. (1996) *Mutat Res.* **359**:171–177.
6. Santella RM, Perera FP, Young TL, Zhang YJ, Chiamprasert S, Tang D, Wang LW, Beachman A, Lin JH, DeLeo VA. (1995) *Mutation Research* **334**:117–124.
7. Chung MK, Riby J, Li H, Iavarone AT, Williams ER, Zheng Y, Rappaport SM. (2010) *Anal. Biochem.* **400**:123-129.

Recent Product Citations

1. Coric, V. M. et al. (2016). Combined GSTM1-Null, GSTT1-Active, GSTA1 Low-Activity and GSTP1-Variant Genotype is associated with increased risk of clear cell renal cell carcinoma. *PLoS One*. doi:10.1371/journal.pone.0160570.
2. Beranek, M. et al. (2016). Genetic polymorphisms in biotransformation enzymes for benzo [a] pyrene and related levels of benzo [a] pyrene-7, 8-diol-9, 10-epoxide-DNA adducts in Goeckerman therapy. *Toxicol Lett.* **255**:47-51.
3. Yang, J. et al. (2016). Ubiquitin protein ligase Ring2 is involved in S-phase checkpoint and DNA damage in cells exposed to benzo [a] pyrene. *J Biochem Mol Toxicol*. doi:10.1002/jbt.21811.
4. Peters, R. E. et al. (2016). Do biomarkers of exposure and effect correlate with internal exposure to PAHs in swine?. *Biomarkers.* **5**:1-9.
5. Barhoumi, R. et al. (2014). Effects of fatty acids on benzo [a] pyrene uptake and metabolism in human lung adenocarcinoma A549 cells. *PLoS One.* **9**:e90908.
6. Borska, L. et al. (2014). Oxidative damage to nucleic acids and benzo (a) pyrene-7, 8-diol-9, 10-epoxide-DNA adducts and chromosomal aberration in children with psoriasis repeatedly exposed to crude coal tar ointment and UV radiation. *Oxid Med Cell Longev.* doi:10.1155/2014/302528.
7. Su, Y. et al. (2014). Interaction of benzo[a]pyrene with other risk factors in hepatocellular carcinoma: a case-control study in Xiamen, China. *Ann Epidemiol.* **24**:98-103.
8. Chiu, C. Y. et al. (2014). MOLECULAR TOXICOLOGY: Low-dose Benzo(a)pyrene and its epoxide metabolite inhibit myogenic differentiation in human skeletal muscle-derived progenitor cells. *Toxicol Sci.* 10.1093/toxsci/kfu003.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's

exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.

7758 Arjons Drive

San Diego, CA 92126

Worldwide: +1 858-271-6500

USA Toll-Free: 1-888-CBL-0505

E-mail: tech@cellbiolabs.com

www.cellbiolabs.com

©2014-2016: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.