BPDE ELISA Kit

ARG83612 BPDE ELISA Kit is an Enzyme Immunoassay kit for the quantification of all sample BPDE in tissue/cell lysate samples.

Catalog number: ARG83612

Package: 96 wells

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PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique for detection and quantitation of BPDE in tissue/cell lysate samples. Standards or samples are pipetted into the DNA high-binding onto a microtiter plate. After washing away any unbound substances, a BPDE-DNA Detection is added to each well and incubate. Following a washing to remove unbound substances, HRP-Streptavidin Solution is added to each microplate well and incubated. After washing away any unbound antibody, a Horseradish Peroxidase (HRP) conjugated primary antibody binds to BPDE is added to each well and incubates. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of total BPDE-DNA bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of total BPDE in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, the Standard and Reduced DNA Standard should be aliquoted and stored at-20°C to avoid repeated freeze-thaw cycles. Store all other components at 4°C. Use the kit before expiration date.

Component	Quantity	Storage information
DNA high-binding microplate	12 X 8 strips	4°C
Standard (0.1 mg/mL BPDE-DNA)	30 µl	-20°C
Reduced DNA Standard (0.2 mg/mL reduced DNA)	200 µl	-20°C
DNA Binding Solution	6 mL	4°C
10X Wash Buffer	100 ml	4°C
1000X conjugated-BPDE Antibody	20 µl	4°C
1000X HRP-Streptavidin	50 µl	4°C
Assay Diluent	50 ml (Ready-to-use)	4°C
TMB substrate	12ml (Ready-to-use)	4°C (Protect from light)
STOP solution	12ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Deionized or distilled water
- TE Buffer (10 mM Tris, pH 8.0, 1 mM EDTA)
- Microplate shaker

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, the Standard and Reduced DNA Standard should be aliquoted and stored at -20°C to avoid repeated freeze-thaw cycles. Store all other components at 4°C. Use the kit before expiration date.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended that the standards and samples be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer, mix well. Storage at 2-8°C.
- 1X conjugated-BPDE Antibody: Dilute the antibody immediately before use; dilute the 1000X conjugated-BPDE Antibody concentrate into Assay Diluent to yield 1X conjugated-BPDE Antibody. Do not store diluted solutions.
- 1X HRP-Streptavidin: Dilute the reagent immediately before use; dilute the 1000X HRP-Streptavidin into Assay Diluent to yield 1X HRP-Streptavidin. Do not store diluted solutions.
- Sample: If the assay found samples contain BPDE higher than the highest standard. The samples can be diluted with Assay Diluent and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

  (It is recommended making series dilutions with Assay Diluent for each unknown sample to do pre-test to determine the suitable dilution factor).

- **BPDE standard:** Prepare a series dilution of **BPDE-DNA standards** with **TE buffer** dilute with **TE buffer** at **1:25** to yield **4 µg/ml concentration**; and **reduced DNA standards** dilute with **TE buffer** at **1:50** to yield **0.1 mg/ml concentration**. The **diluted reduced DNA standard** serves as zero standard (0 µg/ml), and the rest of the standard serial dilution can be diluted with **Reduced DNA** as according to the suggested concentration table below:

Standard No.	BPDE-DNA Conc. (ng/mL)	Diluted Reduced DNA (4 µg/ml) (µl)	Diluted BPDE-DNA Standards (4 µg/ml) (µl)
S1	100	390	10
S2	50	200	200 µl (S1)
S3	25	200	200 µl (S2)
S4	12.5	200	200 µl (S3)
S5	6.25	200	200 µl (S4)
S6	3.13	200	200 µl (S5)
S7	1.56	200	200 µl (S6)
S0	0	200	0

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

ASSAY PROCEDURE

Warm Substrate Solution to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microtiter strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it. Standards and samples should be assayed in duplicates.
2. Add **50 µl** of the **Standards** and **samples** into the appropriate wells.
3. Add **50 µl** of the **DNA Binding Solution** into each wells. Incubate **overnight** at **room temperature** on a microplate shaker**.**
4. Aspirate each well and wash, repeating the process 1 times for a total **2 washes**. Wash by filling each well with **PBS (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
5. Add **200 µl** of the **Assay Diluent** to each well, incubate for **1 hour at RT** on a microplate shaker.
6. Aspirate each well and wash, add **100 µl** of the **1X conjugated-BPDE antibody** to each well, incubate for **1 hour at RT** on a microplate shaker.
7. Aspirate each well and wash, repeating the process 4 times for a total **5 washes**. Wash by filling each well with **1X 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
8. Add **100 µl** of the **1X HRP-Streptavidin working solution** to all wells and incubate for **1 hour at RT** on a microplate shaker.

9. Aspirate each well and **wash as step 7.**
10. Add **100 µl** of **TMB substrate solution** into each well. Incubate for **2-30 mins at RT** on microplate shaker. Avoid exposure to light.
11. Add **100 µl** of **Stop Solution** to each well.
12. Read the OD with a microplate reader at **450 nm** immediately.

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. Using 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 details. (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.