Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Bisphenol A ELISA Kit

07/19

(Catalog # E4722-100; 96 assays, Store kit at -20°C)

I. Introduction:

Bisphenol A (BPA) is a synthetic organic compound and the main starting material used for synthesis of polycarbonate plastics and epoxy resins. Polycarbonate plastics are often used in containers that store foods and beverages such as water bottles while epoxy resins are widely used to coat the inside of metal products such as food cans, bottle tops and water pipelines. These plastics are so universal now but they may degrade to leach BPA into the foods or liquids after long-term exposure. As BPA belongs to an endocrine disruptor compound and can bind to estrogen receptors to induce estrogen-mimicking hormone-like properties, the ingestion of BPA may pose potential dangers to animals or humans. Many studies have suggested the harmful effects of BPA including obesity, infertility, heart disease, diabetes, breast and prostate cancers, as well as neurological and thyroid disorders. As a result, the determination of BPA levels in humans and animals is important in different research areas. The traditional techniques/instruments (HPLC or GC-MS) for detecting BPA are expensive, laborious, and time-consuming. On the other hand, immunoassay techniques, such as ELISA, are commonly preferred as simple, reliable and rapid methods. BioVision's BPA ELISA kit is a competitive-based ELISA that can detect BPA in urine and serum samples. It can detect and quantify BPA (0.4 – 30 ng/ml) within 120 minutes.

II. Applications:

In vitro, quantitative determination of BPA
Detection Range: 0.4- 30 ppb
Sensitivity: 0.2 ppb

III. Sample Type:

Serum and urine

IV. Kit Contents:

Components	E4722-100	Cap Code	Part Number
ELISA Microplate	8 X 12 Strips	--	E4722-100-1
BPA Standard	2 vials	Yellow	E4722-100-2
HRP Conjugate Stock	20 µl	Blue	E4722-100-3
Antibody	7 ml	NM/Red	E4722-100-4
TMB substrate	12 ml	Amber	E4722-100-5
Stop Solution	10 ml	NM/Blue	E4722-100-6
Wash Buffer (10X)	50 ml	NM	E4722-100-7
Sample Diluent	20 ml	NM	E4722-100-8
Serum Solution	2 ml	Brown	E4722-100-9
Standard Buffer	40 ml	WM	E4722-100-10
Conjugate Buffer	7.5 ml	NM/Green	E4722-100-11
Plate Sealers	4	--	E4722-100-12

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 and 650 nm
- Precision pipettes with disposable tips
- Clean eppendorf tubes for preparing standards and sample dilutions

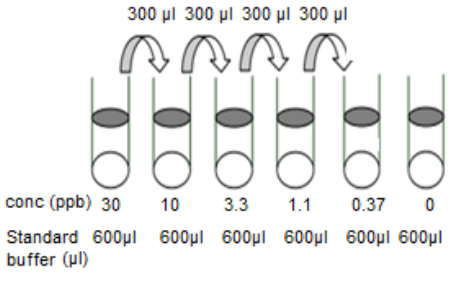
VI. Storage and Handling:

The entire kit may be stored at -20°C for up to 12 months from the date of shipment. Opened kit is stable for 2 months at 4°C.

VII. Reagent and Standard Preparation:

Bring all reagents to room temperature before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- **Antibody, TMB Substrate, Stop Solution, Sample Diluent, Serum Solution, Standard Buffer and Conjugate Buffer:** Ready to be used. After use, store them at 4°C.
- **HRP Conjugate Stock**: Spin briefly before opening the tube. Pipet 12 µl of HRP Conjugate Stock into Conjugate Buffer (7.5 ml) bottle to prepare conjugate working solution. Vortex the bottle for a minute. The conjugate working solution is stable at 4°C for 2 months.
- **Wash Buffer (10X)**: Bring bottle to room temperature. If crystals are present, warm up to room temperature and mix gently until the crystals are completely dissolved. Prepare 100 ml of 1X Wash Buffer by diluting 10 ml of Wash Buffer (10X) with 90 ml deionized water. The 1X solution can be stored at 4°C for one month.
- **BPA Standards**: Reconstitute the BPA standard by adding 1 ml of Standard Buffer to prepare 60 ppb standard stock solution. Allow it to sit at room temperature for 10 minutes and then gently vortex to mix completely. To prepare the 30 ppb standard (S5), mix equal volume of the standard stock and Standard Buffer. Perform 3-fold serial dilutions from S5 (e.g. add 300 µl standards in 600 µl Standard Buffer) to prepare standards S4 to S1 sequentially. The recommended standard dilutions are: 30, 10, 3.3, 1.1, 0.37 and 0 ppb. S0 is the Standard Buffer only. These standards are stable at -20°C for up to 3 weeks.



VIII. Sample Preparation:

- **Serum**

1. Add 20 µl of Serum Solution into 180 µl of serum in an Eppendorf tube and vortex well. Incubate samples at 37°C for 45 min.
2. After the first incubation, incubate samples at 85-90°C for 10 min.
3. After 10 min, dilute the serum sample 40-fold using the Sample Diluent (For example, mix 10 µl of serum with 390 µl of Sample Diluent.)
4. Use 50 µl per well for the assay.
   Note: Dilution factor: 40

- **Urine**

1. Centrifuge 0.5 ml of urine at 10,000 x g for 5 min and collect the supernatant.
2. Dilute the supernatant 40-fold with Sample Diluent (For example, mix 10 µl of urine with 390 µl of Sample Diluent.)
3. Use 50 µl per well for the assay.
   Note: Dilution factor: 40

IX. BPA ELISA Assay Protocol:

Notes: We recommended that all standards and samples are run in duplicate. A Standard curve must be run each time an assay is performed.

1. Prepare all reagents, standards and samples as sections VII and VIII specify respectively.
2. Add 50 µl of **Standards or Samples** per well. Then add 50 µl of **conjugate working solution** and 50 µl of **Antibody** to the above wells.
3. Cover the microtiter plate with plate sealer and mix well. Incubate the plate at room temperature (25°C) for 60 min.
4. Aspirate all reagents and wash each well 5 times: add 250 µl of **1X Wash Buffer** and incubate for 30 seconds. Remove 1X Wash buffer completely before the next wash. (Complete removal of wash buffer is essential for accurate results.) Repeat wash step 4 more times.
5. Add 100 µl of **TMB Substrate** to each well. Tap or shake the plate to ensure complete mixing.
6. Check the OD at 650 nm for the well containing no BPA (S0). When its reading is approximately between 0.85 and 1 (usually between 5-30 min after adding the TMB Substrate), add 50 µl of **Stop Solution** and gently tap the plate to ensure thorough mixing.
7. Measure the OD at 450 nm.

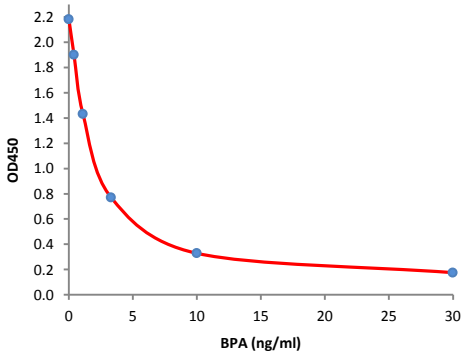
X. Calculation:

The mean values of relative absorbance are divided by the absorbance value of the zero-standard (A_0) and multiplied by 100%. The zero-standard is set to 100% and the relative absorbance of the standards and samples (A) are expressed as percentages.

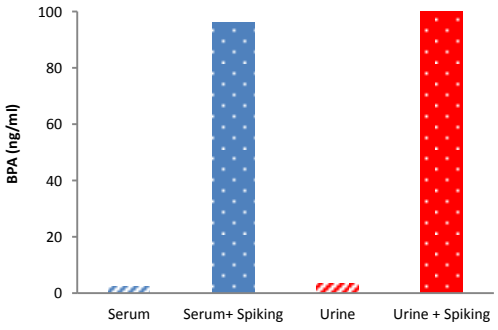
Relative Absorbance (%) = A/A_0 X 100%
A: The average absorbance of the standards or samples
A_0 : The average absorbance of the zero standard

The Standard Curve is done by plotting the relative absorbance of the standards vs. BPA concentrations. The concentration of BPA of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor.

A.



B.



Figures. **A.** BPA standard curve (*This standard curve is for demonstration only. A standard curve must be run with each assay*). **B.** Spike recovery experiment: Human serum and urine samples were assayed with and without spike (100 ng/ml) and showed 90-100% recovery.

XI. RELATED PRODUCTS:

Gentamicin (serum/urine) ELISA Kit (Cat. No. K4315-100)
Folic Acid ELISA Kit (Cat. No. E4523-100)
Caffeine Acid ELISA Kit (Cat. No. E4558-100)
His-Tag Protein ELISA Kit (Cat. No. E4550-100)
DYKDDDDK-Tag Protein ELISA Kit (Cat. No.E4700-100)
Ampicillin ELISA Kit (Cat. No. E4350-100)
Quinolone ELISA Kit (Cat. No. E4530-100)
Vancomycin ELISA Kit (Cat. No. E4605-100)
GST Tag ELISA Kit (Cat. No. E4690-100)

FOR RESEARCH USE ONLY! Not to be used on humans.