ELISA kits available from ADI (see details at the web site)

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	**#1200**	Human Albumin (Urinary)
#1750	Human IgG (total)	**#1760**	Human IgM
#1800	Human IgE	**#1810**	Human Ferritin
#1210	Human Transferrin (Tf)	**#0020**	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		

#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	**#1830**	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	**#1510**	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		

#0030	Human Insulin	**#0040**	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	#**0410**	HCG-free beta

#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	**#1110**	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	**#1700**	Human T3 (total)

#1850	Human Cortisol	**#1860**	Human Progesterone
#1865	Human Pregnolone	**#1875**	Human Aldosterone
#1880	Human Testosterone	**#1885**	Human free Testosterone
#1910	Human Androstenedione	**#1920**	Human Estradiol
#1925	Human Estrone	**#1940**	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3410	Human serum Neopterin		

#3000	Human Rheumatoid Factors IgM (RF)
#3100	Human anti-dsDNA
#3200	Anti-Nuclear Antibodies (ANA)

Instruction Manual No. M-EE-210010

Bisphenol A (BPA) ELISA KIT
Cat. # EE-210010, 96 Tests

For detection of bisphenol A (BPA) in environmental samples

For In Vitro Research Use Only (RUO)

ALPHA DIAGNOSTIC INTERNATIONAL

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LIFE TECH Life Technologies™
Your Molecular & Cell Technology Partner

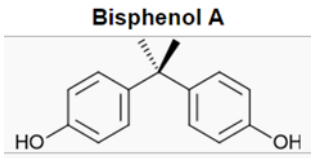
Bisphenol A (BPA) ELISA KIT Cat. No. EE-210010 (96 tests)

Kit Components (96 tests)	Cat #
BPA coated Strip plate, (96 wells)	210011P
BPA **Std. A**, 0 µg/mL, 2 X 0.75 mL	210012A
BPA **Std. B**, 0.01 µg/mL, 2 X 0.75 mL	210012B
BPA **Std. C**, 0.05 µg/mL, 2 X 0.75 mL	210012C
BPA **Std. D**, 0.2 µg/mL, 2 X 0.75 mL	210012D
BPA **Std. E**, 1 µg/mL, 2 X 0.75 mL	210012E
BPA **Std. F**, 5 µg/mL, 2 X 0.75 mL	210012F
Anti-BPA polyclonal chicken antibody, **251X**, 0.10 mL	210013
Anti-IgY-Px enzyme **Conjugate**, **251X**, 0.10 mL	210014
Dilution buffer, 60 ml	210015
Wash buffer (10X), 55 ml	WB-10
TMB Substrate Solution, 13 ml	TMB-13
Stop Solution, 13 ml	ST-13
Complete Instruction Manual	**M-EE-210010**

Intended Use

ADI Bisphenol A (BPA) ELISA Kit is intended for the detection of bisphenol A (BPA) in environmental samples. This kit is for **in vitro research use only (RUO).**

Introduction - The bisphenols are a group of chemical compounds with two hydroxyphenyl functionalities. Most of them are based on diphenylmethane. Bisphenol A is the most popular representative of this group, often simply called "bisphenol" (with 2 hydroxyphenyl groups). BPA is employed to make certain plastics and epoxy resins. BPA-based plastic is clear and tough and is made into a variety of common consumer goods, such as water bottles, sports equipment, CDs, and DVDs. Epoxy resins containing BPA are used to line water pipes, as coatings on the inside of many food and beverage cans and in making thermal paper such as that used in sales receipts. In Japan, more than 0.48 million tons of BPA are produced each year and the water survey report from ministry of the environment says that there is 0.11 µg/L of BPA in rivers. BPA exhibits estradiol-like properties that raise concern about its suitability in some consumer products and food containers. USA, the European Union and Canada have banned BPA use in baby bottles. World production capacity of this compound was more than 2.2 million tons in 2009. The CDC had found bisphenol A in the urine of 95% of adults sampled in 1988–1994[48] and in 93% of children and adults tested in 2003–04. While the EPA considers exposures up to 50 µg/kg/day to be safe, the most sensitive animal studies show effects at much lower doses, and several studies of children, who tend to have the highest levels, have found levels over the EPA's suggested safe limit figure.



PERFORMANCE CHARACTERISTICS

Intra-assay precision CV :<3%

Inter-assay precision CV: <9%

Measuring range

0.01 ug/mL-1.0 ug/mL

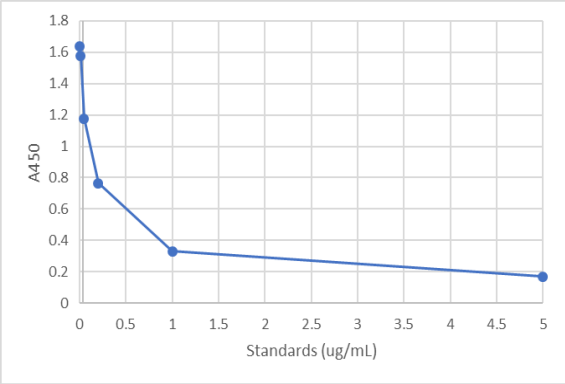
Detection Limit

10 ng/mL

WORKSHEET OF A TYPICAL ASSAY

Wells	Stds/samples	Mean A450
A1, A2	**Std. A** (0 ug/mL)	1.639
B1, B2	**Std. B** (0.01 ug/mL)	1.579
C1, C2	**Std. C** (0.05 ug/mL)	1.178
D1, D2	**Std. D** (0.2 ug/mL)	0.764
E1, E2	**Std. E** (1 ug/mL)	0.331
F1, F2	**Std. F** (5 ug/mL)	0.169
	SAMPLE	1.00

NOTE: These data are for demonstration purpose only.



CALCULATION OF RESULTS

1. Subtract the absorbance of the background (absorbance of the Dilution buffer + methanol well) from the absorbances of all other wells.
2. Calculate the average values of absorbances of standards.
3. Construct the standard curve by plotting the absorbance (Y-axis) versus log of the BPA concentration (X-axis).
4. Read the concentration of the unknowns from the standard curve.

Quality Control

The test results are only valid if the test has been performed following the instructions. All standards and kit controls must be found within the acceptable ranges as stated on the vials. If criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. In case of any deviation the following technical issues should be proven (reagents, protocol, equipments, etc).

PRINCIPLE OF THE TEST

Bisphenol A ELISA kit is based on competitive binding of BPA present in the sample and BPA immobilized to the wells of the microtitrate plate for binding sites of the anti-BPA polyclonal chicken antibody. The bound antibodies are detected with anti-chicken antibody peroxidase conjugate anti-IgY Px. BPA present in the sample is determined by a color reaction with the chromogenic substrate (TMB). The enzymatic reaction (blue color) is inversely proportional to the amount of BPA concentration in the sample. The reaction is terminated by adding stopping solution (converts blue to yellow). Absorbance is then measured on an ELISA reader at 450 nm and the concentration of BPA in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipette (25-100 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, Orbital shaker, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

ADI Bisphenol A ELISA test is intended for *in vitro research* use only. Chromogenic substrate (TMB substrate), Diluent buffer and Standards contain preservative ProClin 300® (mix of 5-Chloro-2-methyl-4-isothiazolin-3-one a 2-Methyl-2H-isothiazol-3-one (3:1)).

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site. TMB (substrate), Diluted H2SO4 (1N, stop solution).

SAMPLE PREPARATION

Use some routine method that is used for the environmental sample extraction in relation to the other method of detection (HPLC or GC).

Note: The extract must be diluted in methanol, dilute the extract with Dilution buffer to get the final concentration of methanol in the tested mixture 10% v/v

Prepare mixture of the diluted sample with anti-BPA antibody by diluting the anti-BPA antibody 251 times with an appropriate volume of the diluted sample – e.g. 150 µL for 1 well – mix 150 µL of the diluted sample with 0.6 µL of anti-BPA antibody.

REAGENTS PREPARATION

Vortex samples, Dilution buffer and TMB substrate in order to ensure homogeneity and mix all solution well prior use

10% Methanol: Prepare 10% methanol solution in Dilution buffer – to determine the background of the reaction—e.g. 100 µL for 1 well – mix 20 µL of methanol with 180 µL of Dilution buffer.

BPA standards: Prepare mixtures of the BPA standards (Standard A, B, C, D, E, F) with the anti-BPA antibody by diluting anti-BPA antibody 251 times in each standard solution– i.e. at least 250 µL for 2 wells – mix 250 µL of a standard with 1 µL of anti-BPA antibody.

Wash buffer (10X): Prepare Wash buffer by diluting the Wash buffer concentrate 10 times with an appropriate volume of distilled or deionized water (e.g. 50 mL of the concentrated Wash buffer + 450 mL of distilled water). If there are crystals of salt present in the concentrated Wash buffer, warm up the vial to +32 to +37°C in a water bath. Diluted Wash buffer is stable for one month if stored at room temperature.

Anti-IgY Px enzyme conjugate: Dilute anti-IgY Px enzyme conjugate 251 times with Dilution buffer – e.g. 0.04 mL anti-IgY Px + 10 Ml Dilution buffer –you need approximately 12 mL of the diluted anti-IgY Px for the whole microplate.

Do not store the diluted samples and the diluted anti-IgY Px conjugate. Always prepare fresh.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{o}C until the expiration date printed on the label. The whole kit stability is approx.. 12 months or as labeled under appropriate storage conditions. The unused portions of the standards should be stored at 2-8^{o}C or stored frozen in small aliquots.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4^{o}C. Do not touch the bottom of the wells.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.

1. Label or mark the microtiter well strips to be used on the plate.
2. Pipet **100 ul of standards**, and samples into appropriate wells in *duplicate*. For blank, add 100 µL of Dilution buffer with methanol.
3. Cover the plate, mix gently for 5-seconds and incubate at room temp for 60 min.
4. Aspirate the well contents and blot the plate on absorbent paper. Immediately, **wash the wells 5 times** with 250 ul of 1X wash buffer. We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
5. Add **100 ul diluted anti-IgY Px-enzyme conjugate** to all well. Mix gently for 5-10 seconds. Cover the plate and incubate for **60 minutes** at room temp.
6. Wash the wells 5 times as in step 3.
7. Add **100 ul TMB substrate solution**. Mix gently for 5-10 seconds. Cover the plate and incubate for **15 minutes** in dark at room temp. **Blue color** develops in positive controls and samples.
8. Stop the reaction by adding **100 ul of stop solution** to all wells. Mix gently for 5-10 seconds to have uniform color distribution (blue color turns yellow).
9. Measure the **absorbance at 450 nm** using an ELISA reader.