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ELISA-VIDITEST Bisphenol A

0DZ-041

Instruction manual

1. TITLE:

ELISA-VIDITEST Bisphenol A

2. INTENDED USE:

- ELISA-VIDITEST Bisphenol A is intended for the detection of bisphenol A (BPA) in environmental samples.
- The measuring range of the test is from 0.01 µg/ml to 1 µg/ml
  (detection limit is 10 ng /ml).
- The test procedure is simple, total time of the assay is 2.25 hour.
- The microtitrate plate with 96 test wells allows measuring a large set of samples simultaneously.

3. TEST PRINCIPLE:

a) competitive reaction

ELISA-VIDITEST Bisphenol A is based on the competition 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). If the samples contain low concentrations of BPA, more molecules of anti-BPA antibody are available for binding to the immobilized BPA and the resulting colour of the wells is dark yellow. If the samples contain high concentrations of BPA, less molecules of the anti-BPA antibody bind to the immobilized BPA and the colour of wells is light yellow-white. The colour development is inversely proportional to the BPA concentration in the sample.

b) quantitative detection

The standard curve is constructed by plotting the absorbance of standards at 450 nm (Y-axis) versus log of the BPA concentrations in µg/ml present in the standards (X-axis). The unknown BPA concentration in the samples is calculated from the standard curve.

4. MATERIAL PROVIDED FOR 96 DETERMINATIONS:

	component	amount	number	storage
1	8-well break-away strips coated with BPA, within a plastic frame STRIPS	12 strips, 96 wells	1 piece	2 – 10 °C
2	BPA STANDARD 0 0 µg/ml r.t.u. **	0.75 ml	2 vials	2 – 10 °C
	BPA STANDARD 1 0,01 µg/ml r.t.u.**			
	BPA STANDARD 2 0,05 µg/ml r.t.u.**			
	BPA STANDARD 3 0,2 µg/ml r.t.u. **			
	BPA STANDARD 4 1 µg/ml r.t.u. **			
	BPA STANDARD 5 5 µg/ml r.t.u. **			
3	anti-BPA polyclonal chicken antibody, 251x conc. anti-BPA 251x	0.10 ml	1 vial	2 – 10 °C
4	anti-IgY Px enzyme conjugate, 251x conc. CONJ 251x	0.10 ml	1 vial	2 – 10 °C
5	Dilution buffer DIL , r.t.u. *	60 ml	1 vial	2 – 10 °C
6	Wash buffer concentrate, 10x concentarted WASH 10x	55 ml	1 vial	2 – 10 °C
7	Chromogenic substrate (TMB substrate) TMB , r.t.u. *	13 ml	1 vial	2 – 10 °C
8	Stop solution STOP , r.t.u. *	13 ml	1 vial	2 – 10 °C
9	Instruction manual		1 piece	
10	Quality control certificate		1 piece	
11	Material Safety Data Sheet		1 piece	

* ready to use

** ready to use, component B -  - contains 10% methanol (see Material Safety Data Sheet)

5. MATERIAL REQUIRED BUT NOT PROVIDED WITH THE KIT:

- Distilled or deionised water for dilution of the Wash buffer concentrate.
- Appropriate equipment for pipetting, liquid dispensing and washing.
- Spectrophotometer/colorimeter (microplate reader – wavelength 450 nm).
- The material required for pre-treatment of environmental samples
- Methanol p.a.

6. PREPARATION OF REAGENTS AND SAMPLES:

a. **Allow all kit components to reach room temperature.**
b. **Vortex samples, Diluting buffer (DIL) and TMB substrate in order to ensure homogeneity** and mix all solution well prior use.
c. Prepare 10% methanol solution in DIL (DIL+M) – to determine the background of the reaction– – **e.g. you need 100 µl for 1 well – mix 20 µl of methanol with 180 µl of DIL.**
d. Prepare mixtures of the BPA standards with the anti-BPA antibody by diluting anti-BPA antibody 251 times in each standard solution– **i.e. you need at least 250 µl for 2 wells – mix 250 µl of a standard with 1 µl of anti-BPA antibody.**
e. Samples: Use some routine method that you use for the environmental sample extraction in relation to the other method of detection (HPLC or GC). **Note: The extract has to be diluted in methanol, dilute the extract with DIL to get the final concentration of methanol in the tested mixture 10% v/v.**
f. 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. you need 150 µl for 1 well – mix 150 µl of the diluted sample with 0.6 µl of anti-BPA antibody.**
g. Prepare Wash buffer by diluting the Wash buffer concentrate 10 times with an appropriate volume of distilled or deionised 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 week if stored at +2 to +10˚C.
h. Dilute anti-IgY Px enzyme conjugate 251 times with DIL – **e.g. 0.04 ml anti-IgY Px + 10 ml DIL –you need approximately 12 ml of the diluted anti-IgY Px for the whole microplate.**
i. Do not dilute TMB substrate and Stop solution, they are ready to use.

7. ASSAY PROCEDURE:

a. Allow the BPA coated strips to reach room temperature before opening to prevent water condensation within the wells. Withdraw an adequate number of strips and put the remaining strips back in the aluminium pouch and seal, keep the desiccant inside.
b. Pipette 100 µL of DIL with methanol, the mixtures of standards and the mixtures of samples with anti-BPA antibody to the wells according to the pipetting scheme (*Figure 1 on page 4*): Fill the first well with DIL+M to determine the reaction background. Fill the next two wells with 100 µL of prepared mixture of standard 0 and then fill wells with the remaining mixtures of standards (1,2...5). Fill the other wells with the mixtures of samples.
c. Incubate for **60 (+/- 5) minutes** at room temperature.
d. Aspirate the liquid from the wells into a collecting bottle (*see Safety Precautions*). Then wash the wells five times with 250 µl/well of Wash buffer. Avoid cross-contamination between wells! If some liquid remains in the wells, invert the plate and tap it on an adsorbent paper to remove the last remaining drops.
e. Pipette 100 µL of the diluted anti-IgY Px-enzyme conjugate in all wells.
f. Incubate for **60 (+/- 5) minutes** at room temperature.
Aspirate the liquid from the wells into a collecting bottle (*see Safety Precautions*). Wash and aspirate the wells five times with 250 µl/well of Wash buffer.
g. Dispense 100 µL of the TMB substrate into each well.
Pipette in a regular rhythm or use an appropriate dispensing instrument.
h. Incubate for **10 minutes (+/-5 seconds)** at room temperature.
The time measurement must be started at the beginning of TMB dispensing.

Cover the strips with an aluminium foil or keep them in the dark during the incubation with TMB substrate.

i. Stop the reaction by adding 100 µL of Stop solution.
Use the same pipetting rhythm as with the TMB substrate to keep the same reaction time in all wells. Tap gently the microplate for a few times to ensure complete mixing of the reagents.

j. Read the absorbance at 450 nm with a microplate reader **preferably immediately**. It is recommended to use a reference reading at 630 nm.

Figure 1: Pipetting scheme

	1	2	3	4	5	6	7	8	9	10	11	12
A	DIL+M	**ST3**	S…									
B	**ST0**	**ST4**										
C	**ST0**	**ST4**										
D	**ST1**	**ST5**										
E	**ST1**	**ST5**										
F	**ST2**	S1										
G	**ST2**	S2										
H	**ST3**	S3										

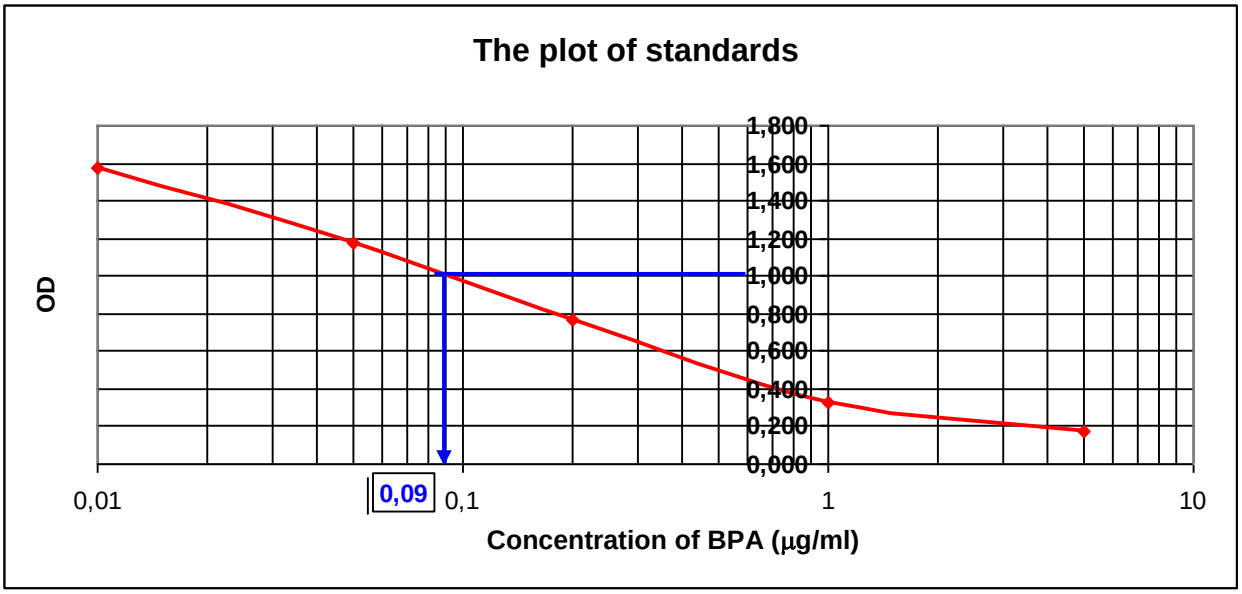
8. PROCESSING OF RESULTS:

a. First subtract the absorbance of the background (absorbance of the DIL+M well) from the absorbancies of all other wells.
b. Calculate the average values of absorbancies of standards.
c. Construct the standard curve by plotting the absorbance (Y-axis) versus *log* of the BPA concentration (X-axis).
d. Read the concentration of the unknowns from the standard curve.

Example:

BPA (µg/ml) standards	**absorbance**
0	1.639
0.01	1.579
0.05	1.178
0.2	0.764
1	0.331
5	0.169

sample	
BPA (µg/ml)	**absorbance**
read from the graph **0.09**	1.000



9. VALIDITY OF THE TEST:

The results of the test are valid if:

- The mean absorbance of mixture DIL+M is less than 0.050.
- The mean absorbance of Standard 5 (ST 5) is more than 0.050.
- The mean absorbance of Standard 0 (ST 0) is more than 1.000.
- The mean absorbencies of Standard can be lined up as follows:
  ST 0 > ST 1 > ST 2 > ST 3 > ST 4 > ST 5

10. SAFETY PRECAUTIONS:

All ingredients of the kit are intended for laboratory use only.

Handle the liquid waste in the collecting bottle, the used strips, BPA standards, the anti-BPA antibody and the anti-IgY Px as the hazardous waste.

Liquid wastes containing acid (Stop solution) should be neutralized in 4% sodium bicarbonate solution. Handle Stop solution with care. Avoid contact with skin or mucous membranes. In case of contact with skin, rinse immediately with plenty of water and seek medical advice.

The environmental samples might contain carcinogens and mutagens (class 2 or 3), handle them as hazardous waste.

Do not smoke, eat or drink during work. Do not pipette by mouth. Wear disposable gloves while handling reagents and samples. Wash your hands thoroughly afterwards. Avoid spilling or producing aerosol.

11. HANDLING PRECAUTIONS:

Avoid contamination of samples and kit reagents.

Avoid cross-contamination of reagents.

Chromogenic substrate (TMB substrate) contains preservative ProClin 300®.

Avoid contact of the TMB substrate with oxidizing agents or metal surfaces.

Follow the assay procedure indicated in the Instruction manual.

Variations in test results are usually due to:

* Insufficient mixing of reagents and samples
* Inaccurate pipetting and inadequate incubation times
* Poor washing technique or spilling the rim of wells with the sample.
* Use of identical pipette tip for different solutions

12. STORAGE AND EXPIRATION DATE:

Store the kit reagents at +2 to +10°C, in a dry place and protected from the light. Avoid freezing. Expiration date is indicated at the kit label and at all reagent labels.

Store unused strips in the sealable pouch and keep the desiccant inside. Transported in thermo bags, upto 72 hours of the transport time has no influence to the expiration. If you find any damage to the kit or the kit reagents advise to the producer.

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

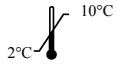
13. USED SYMBOLS:

Number of wells


Manufacturer


Expiration

Lot of kit



Storage at +2°C - +10°C


Read usage instructions

14. FLOW CHART:

Step 1.	**Prepare reagents and the mixtures of standards and of the samples with the anti-BPA antibody**
	↓
Step 2.	**Dispense 100 µL/ well of the standard mixtures and of the sample mixtures**
	↓
	Incubate 60 min at room temperature
	↓
	Wash 5 times (250 µL/ well), aspirate
	↓
Step 3.	**Dispense 100 µL/ well of anti-IgY-Px conjugate**
	↓
	Incubate 60 min at room temperature
	↓
	Wash 5 times (250 µL/well), aspirate
	↓
Step 4.	**Dispense 100 µL/well TMB substrate**
	↓
	Incubate 10 min at room temperature, dark
	↓
Step 5.	**Dispense 100 µL/well of Stop solution**
	↓
Step 6.	**Read optical density for 450 nm (ref. 630nm) preferably immediately**

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