Code No. 27766

Bisphenol A (BPA) Assay Kit - IBL

INTRODUCTION

Bisphenol A (BPA), principal raw material for epoxy resins and polycarbonate is suspected as one of the endocrine disrupting chemicals. BPA is a chemical building block for making polycarbonate plastic used for food containers and feeding bottles, which touches mouths directly, and is also used in production of epoxy resins for food and beverage can linings. In Japan, more than 0.48 million tons of BPA are produced each year and the water survey report from ministry of the wnvironment says that there is 0.11μg/L of BPA in rivers. So, we are anxious about the adversely impact for aquatic organisms and ecosystems. Vom Saal reported that in pregnant mice dosed at 2 μg per kg of body weight with a level of 1/25 of the threshold limit value per day, the prostate of the male offspring was enlarged.
This product is made for research use only by measuring BPA in serum of plasma; it is based on an ELISA using anti-rabbit IgG antibody coated solid-phase method.

PRINCIPLE

This kit is based on a competitive ELISA using anti-rabbit IgG antibody solid-phase method. BPA standard or sample is taken on a anti-rabbit IgG antibody coated microplate and enzyme-labeled BPA and anti-BPA serum are added to cause a reaction. After the reaction is completed, the plate is washed and the enzyme reaction is allowed to proceed with addition of a substrate. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The absorbance is then measured by using a plate reader. A standard curve is draw, and the BPA concentration in the sample is obtained.

MEASUREMENT RANGE

0.3 ～ 100 ng/mL

INTENDED USE

This IBL's assay kit is capable for the quantitative determination BPA in human serum and plasma.

KIT COMPONENT

1	Precoated plate	: Anti-Rabbit IgG Antibody	96 Well x 1
2	Labeled BPA	: HRP conjugated BPA	6 mL x 1
3	Standard : BPA (0 ng/mL, 0.3 ng/mL, 1 ng/mL, 3 ng/mL, 10 ng/mL, 30 ng/mL, 100 ng/mL)		0.3 mL x 7
4	Anti-BPA Antiserum		6 mL x 1
5	Unused number		
6	Chromogen	: TMB solution	15mL x 1
7	Stop solution	: 1N H_2SO_4	12mL x 1
8	Wash buffer Conc.	: (40X) 0.05% Tween20 in phosphate buffer	50mL x 1

OPERATION MANUAL

1. Materials needed but not supplied

- Plate reader (450nm)
- Micropipette and tip
- Graduated cylinder and beaker
- Deionized water
- Plate mixer
- Graph paper (semi-logarithmic)
- Paper towel
- Washing bottle for precoated plate
- Disposable test tube for "6, Chromogen"

2. Preparation

Preparation of wash buffer
"8, Wash buffer Conc." is a concentrated (40X) buffer. Adjust the temperature of "8, Washing buffer Conc." to room temperature and then, mix it gently and completely before use. Dilute 50 mL of "8, Wash buffer Conc." with 1,950 mL of deionized water and mix it. This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.

The other reagents are ready for use.

3. Measurement procedure

All reagents shall be brought to room temperature before use. Then mix it gently and completely before use. Make sure of no change in quality of the reagents.
Standard curve shall be prepared simultaneously with the measurement of test samples.
Usually, duplicate measurement shall be conducted.

Hereinafter, refer to Fig. 1 and Table 1.

1) Prepare necessary amounts of "1, Precoated plate" strips for measurement. 16 wells (2 strips) for preparation of the standard curve including blanks and 2 wells for measuring one sample are needed.
2) Add 20μL of "3, Standard" (0 ng/mL) into well A-1 and A-2, and add "3, Standard" (0.3 ng/mL) into wells B-1 and B-2. In the same manner, add each concentration "3, Standard" into wells C-1, C-2, D-1, D-2, E-1, E-2, F-1, F-2, G-1 and G-2. (Leave wells H-1 and H-2 open for Blanks.)
Add 20μL of test sample into well of 3rd – 12th column.
3) Add 50μL of "2, Labeled BPA" to each of the wells into which the standard or sample was put. Also, add 50μL of "4, Anti-BPA Antiserum" to each of the wells into which the standard or sample was put.
4) Seal the precoated plate and stir it for 5 minutes with the plate mixer, and then incubate the precoated plate for 55 minutes at room temperature (20–30 °C).
5) Wash each well of the precoated plate vigorously with wash buffer using the washing bottle. Then, fill each well with wash buffer and leave the precoated plate laid for 15-30 seconds. Remove wash buffer completely from the precoated plate by snapping. This procedure must be repeated more than 7 times. Then, remove the remaining liquid from all wells completely by snapping the precoated plate onto paper towel.
In case of using a plate washer, after 4 times washing with plate washer, washing with above washing bottle must be repeated 3 times.
6) Take the required quantity of "6, Chromogen" into a disposable test tube. Then, pipette 100 μL from the test tube into the wells. Please do not return the rest of the test tube to "6, Chromogen" bottle to avoid contamination.
7) Incubate the precoated plate for 30 minutes at room temperature in the dark.
8) Add 100 μL of "7, Stop solution" to all wells. Mix the liquid by tapping the side of precoated plate. The liquid will turn yellow from blue by addition of "7, Stop solution".
9) Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the liquid. Then, run the plate reader and conduct measurement at 450 nm against one of Blanks (H-1 or H-2) within 30 minutes after addition of "7, Stop solution".

Fig. 1

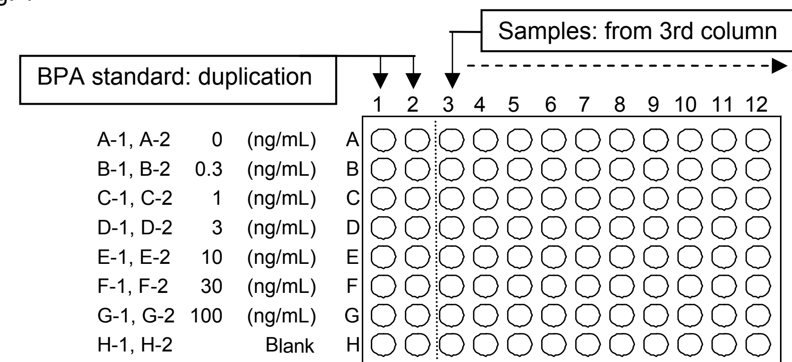

Table 1

	Standard	Test samples	Blank
Sample	Standard 20 μL	Test sample 20 μL	–
Labeled BPA	50 μL	50 μL	–
Anti-BPA Antiserum	50 μL	50 μL	–
Mix for 5 minutes by plate mixer after sealing plate. Incubate for 55 minutes at room temperature (20-30°C).			
Washing 7 times			
Chromogen	100 μL		
Incubate for 30 minutes at room temperature (shielded).			
Stop solution	100 μL		
Tap the plate for mixing, and then read the plate at 450nm against a blank within 30 minutes after addition of Stop solution.			

SPECIAL ATTENTION

1) Use serum or plasma as a sample. When samples are to be stored, keep them in a cool place or at -20°C.
2) Duplicate measurement of test samples and standard is recommended.
3) Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
4) Use only wash buffer contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
5) Remove the wash buffer completely by tapping the precoated plate on paper towel. Do not wipe wells with paper towel.
6) "6, Chromogen" should be stored in the dark due to its sensitivity against light. "6, Chromogen" should be avoided contact with metals.
7) Measurement should be done within 30 minutes after addition of "7, Stop solution".

CALCULATION OF TEST RESULT

1) Calculate the average of the absorbance values of "0 ng/mL BPA standard" wells, A-1 and A-2, (B_0).
2) For the other wells, calculate the binding rates to B_0, B/B_0 (%), as following formula.

$$B / B_0 (\%) = \frac{\text{(absorbance of each well)}}{B_0} \times 100$$

3) Plot the value (B/B_0) of each standard on semi logarithmic graph paper, with the concentrations of standard on the logarithmic scale (abscissa) and the binding rates (B/B_0) on the integer scale (ordinate), and draw a standard curve.
4) Obtain the binding rates (B/B_0) of other wells which were used for the samples, in the same way. And read the BPA concentration of samples from the standard curve.

Example of measurement values and standard curve

Conc. (ng/mL)	Absorbance (450nm)	B / B_0 (%)
0	1.828 (B_0)	100.0
0.3	1.554	85.0
1	1.349	73.8
3	1.058	57.9
10	0.685	37.5
30	0.343	18.8
100	0.163	8.9

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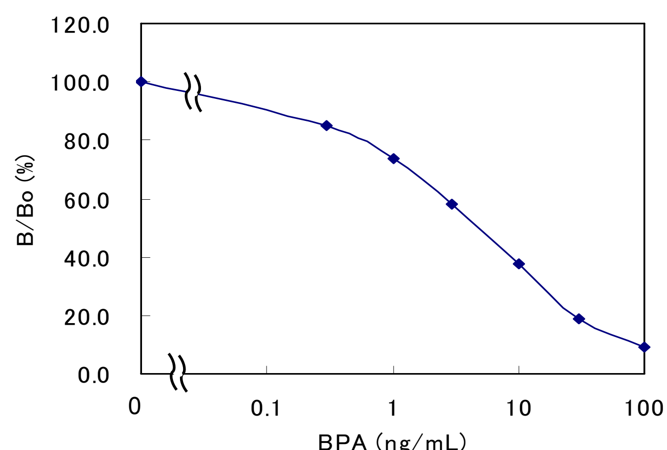

＊ The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

PERFORMANCE CHARACTERISTICS

1. Sensitivity

1) The absorbance of BPA at 0 ng/mL (B_0) is 1.5 or more.
2) The absorbance ratio of standard 100 ng/mL (B_{100}) to standard 0 ng/mL (B_0), (B_{100}/B_0 x100) is less than 20 %.

2. Intra Assay

Measurement Value (ng/mL)	SD value	CV value (%)	n
1.32	0.19	14	8
4.49	0.38	8.5	8
14.73	1.03	7	8
31.15	1.72	5.5	8

3. Specificity

Compoond	Cross-reactivity
BPA	100 %
BPA-Glucronide	85.0 %
BPA-Na-Sulfate	68.0 %
Bisphenol B	8.3 %
Bisphenol F	0.2 %
Dietylstilbestrol	≦ 0.02 %
Hexesterol	
17β-Estradiol	
4-Heptylphenol	
4-n-Nonylphenol	
4-Propylphenol	
4-Hexyloxyphenol	
4-Pentylphenol	
4-Hexylphenol	
4-Butylphenol	
2-ter-Butylphenol	
4-Dodecylphenol	
Di-n-Butyl-Phthalate	
Benzyln-Butyl Phthalate	
Daidzein	
Genistein	
Bis-GMA	

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1. Bring each reagent and samples to room temperature and mix it to homogeneity without foaming.
2. "7, Stop solution" is a strong acid substance. Therefore, be careful not to have your skin and clothes contact "7, Stop solution" and pay attention to the disposal of "7, Stop solution".
3. Since the measurement results are affected by the time and temperature of incubation, the samples and standards must be incubated under the same conditions.
4. "1, Precoated plate" and "3, Standard" contain sodium azide. Therefore, dispose these materials after diluting them with large quantity of water to avoid production of explosive metallic azide.
5. After testing, disposal must be made in accordance with national and local regulation separating the infectious waste.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from a different lot or kit.
8. All reagents should be stored at 2 - 8°C and do not use expired reagents. Put unused precoated plate strips into a sealed bag with a desiccant, and use by expiration date.
9. This kit is for research purpose only. Do not use for clinical diagnosis.
10. Handle samples as if there is capable of infection (HBvirus, HIV). The implements used for the assay should be treated in 0.1 % sodium hypochlorite solution or autoclave.

STORAGE AND THE TERM OF VALIDITY

Storage Condition : 2 - 8°C
The term of validity : 12 months
(The expiry date is specified on outer box.)

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Version 1.