

BPA (Bisphenol A) ELISA kit

Catalog Number: **BPA1**

Store at -20°C.

FOR RESEARCH USE ONLY



Introduction

This competitive ELISA kit is for determination of BPA levels in biological samples. A typical standard curve of the ELISA (**detection limit, <10 pg/ml**) is shown on page 6. BPA is a phenolic environmental estrogen¹ which disrupts endocrine activity. In human, a BPA glucuronide was a primary metabolite of BPA². In a recent study, the age group with highest BPA exposure was 6-11 years old with a mean total (free + glucuronidated) BPA level of 4.33 ng/g of creatinine³. Urinary BPA levels were correlated with cardiovascular diseases and diabetes⁴. A recent study revealed that a 12-ounce serving of canned soup for 5 days increased urinary BPA level 12-fold due to BPA-containing epoxy resin lining of the cans⁵.

This kit can be used for the determination of BPA in urine, serum, plasma, cells, and tissues following proper isolation and purification. Instructions are provided as to the proper isolation and purification in the following pages. The free BPA level in urine or cell culture media can be measured using the BPA ELISA, without ethyl acetate extraction after 4-fold dilution of the sample.

This competitive ELISA kit, based on competition between the BPA epitope and BPA-HRP conjugate for a limited number of binding sites available from the anti-BPA antibody, which is coated on the bottom of the wells of the 96 well ELISA plate. The conjugate concentration is held constant in each well, while the concentration of the BPA is variable, based on the concentration of the sample or standard. Thus, the amount of the BPA conjugate which is able to bind to each of the wells is inversely proportional to the concentration of BPA in the standard or sample. The amount of the conjugate which is bound to each well is then determined by the amount of color obtained, when TMB is added. The TMB reacts with the HRP available in the well. With the addition of sulfuric acid, the blue colored product is converted into a yellow-colored product, which can be read on a plate reader at 450 nm.

Storage and Stability

This kit will obtain optimal results if all of the components are stored at the proper temperature prior to use. Items should be stored at the designated temperatures upon receipt of this kit.

All components are stored below -20°C and should not be re-frozen and thawed more than necessary.

Precautions

- Please read all instructions carefully before beginning the assay.
- The reagents in this kit have been tested and formulated to perform optimally. This kit may not perform correctly if any of the reagents are replaced or any of the procedures are modified.
- This kit is intended for research use only and is not to be used as a diagnostic.

Materials Provided

Part Number	Item	Description	Quantity
1	BPA ELISA Plate	Solid 96-well plate coated with anti-BPA antibody in each well	1
2	BPA Standard (2 µL)	Stock standard at a concentration of 1 mg/mL	1
3	BPA-HRP Conjugates (12 µL)	1000 X concentrated solution	1
4	Sample Dilution Buffer (25 mL)	10 X solution of Tris-buffered saline with preservatives	1
5	HRP Buffer (15 mL)	1 X solution of Tris-buffered saline with preservatives	1
6	Wash Buffer Solution (25 mL)	10 X solution of Tris-buffered saline with detergents and preservatives	1
7	TMB Substrate (24 mL)	A solution of TMB (tetra methyl benzidine)	1

Additional Required Materials (Not Provided)

- An 8-channel adjustable pipette and an adjustable pipette
- Storage bottles
- Costar® cluster tubes (1.2 mL) and microcentrifuge tubes
- Deionized water
- 2N Sulfuric acid

Procedural Notes

- Remove all of the reagents required, including the TMB, and allow them to equilibrate to room temperature before proceeding with the assay.
- It is necessary to thoroughly mix the concentrated buffer solutions. A stir bar is contained within each buffer solution.

Sample Preparations

There are different protocols for isolating BPA depending on the nature of the biological sample. Listed below are the different protocols. For optimal results follow the appropriate protocol based on the biological sample.

BPA measurement in urine

1. Dilute urine sample 4-fold with 1X sample dilution buffer. Centrifuge diluted urine sample to remove any precipitates.

2. Add 100 μL of the sample to a well in the 96-well plate and perform the ELISA for BPA (according to the instructions of the manufacturer).

BPA measurement in water

Add 100 μL of the sample to a well in the 96-well plate and perform the ELISA for BPA according to the instructions of the manufacturer.

BPA measurement in plasma or serum

1. Combine 1.0 mL of plasma (adjusted with acetic acid to pH 4) and 1.0 mL of ethyl acetate. Vortex thoroughly. Centrifuge at 2000 rpm for ten minutes at 22°C. Three phases should result:
 - i. Upper organic phase – ethyl acetate phase (lipoproteins)
 - ii. Interphase – proteins
 - iii. Lower phase – aqueous phase
2. Collect the upper organic phase (a) and set aside.
3. Discard the interphase. Transfer the lower phase with a glass pipette to a new tube, and repeat the ethyl acetate extraction step 2 more times.
4. Evaporation of pooled organic phase: There should be approximately 3 mL of the ethyl acetate phase (a). Dry the pooled organic phase in a speed vac to get the extracted sediment (b).
5. Store the sediment (e) at -20°C if performing assay later. For ELISA assay, dissolve the sediment (e) in 20 μL of ethanol or DMF, then add 130 μL of 1x Sample Dilution Buffer.
6. For the competitive BPA ELISA, the above 150 μL sample may need to be further diluted. When calculating the concentration, consider any dilution factors.
7. Perform the ELISA for BPA (according to the instructions of the manufacturer).

BPA measurement in cells

1. Collect and homogenize and/or sonicate the cells.
2. Acidify the whole homogenized cells with acetic acid to a pH of approximately 3-4. Measure using standard pH paper.
3. Extraction with ethyl acetate. Add an equal volume of ethyl acetate to the homogenized cells and vortex very well. Place the upper organic phase into a fresh clean tube after centrifugation. Then add another equal volume of ethyl acetate to the homogenized cells to start the second-time extraction. It is strongly recommended that extraction is performed three times.
4. Evaporate the pooled ethyl acetate from the extractions until all has dried up under argon or nitrogen gas.
5. Add 10 μL to 20 μL ethanol, or N, N-dimethyl-formamide (DMF), to reconstitute the dried-up residue from above step #4. Add 0.5 mL of 1x Sample Dilution Buffer (provided in kit). Load 100 μL in each well, in triplicates, on the ELISA plate. (Note: We recommend measuring a different dilution of sample in attempt to fit the results to the standard curve. e.g., add 3 wells with 50 μL of the rest of sample plus 50 μL 1x Sample Dilution Buffer, and 3 wells with 10 μL of the rest of sample plus 90 μL of 1x Sample Dilution Buffer.)
6. Perform the ELISA for BPA (according to the instructions of the manufacturer).

BPA measurement in tissues

1. Homogenize 1 g of tissue, 4 mL of H₂O.
2. Acidify the homogenate by adding 8 μL of acetic acid to each homogenate.
3. Extract with an equal amount of ethyl acetate, vortex thoroughly, spin down, and collect the organic phase. Repeat this extraction twice more and combine all of the organic phases.
4. Dry the organic phase with argon or nitrogen gas.
5. Dissolve the dried residue from above step #4 with ethanol or DMF. (Add approximately 20 μL of ethanol or DMF to reconstitute the dried-up residue.)
6. Dilute further with 1x Sample Dilution Buffer: Add approximately 0.5 mL of 1x Sample Dilution Buffer and centrifuge at 10,000 rpm for five minutes at room temperature. The supernatant will be used for ELISA.
7. Perform the ELISA for BPA (according to the instructions of the manufacturer).

BPA measurement in canned foods

BPA in liquid of human canned food can be measured by the following protocol.

1. Strain liquid portion from any solids
2. Measure volume of liquid (or weigh)
3. Transfer liquid to centrifuge tube and spin at 1000g for 4 minutes at 4°C to remove any remaining solids.
4. Save supernatant
5. Dilute supernatant 4-fold (more or less dilution may be required) and add 100 uL of sample to well of ELISA plate.
6. Perform ELISA as described in Instruction Booklet provided with kit

BPA measurement in animal chow

1. Weigh out 6.5 g of each lab chow
2. Pulverize lab chow with porcelain mortar and pestle
3. Placed in 50 mL tubes and added 10 mL HPLC grade water to powdered lab chow
4. Vortex vigorously for 30 seconds
5. Place on platform rocker and shake for 1 hour
6. Sonicate for 20 seconds at 80% full power
7. Vortex vigorously
8. Remove 2.0 mL to new tube
9. Add 3.0 mL of ethyl acetate and vortex
10. Centrifuge at 3000 g for 5 minutes to separate layers
11. Remove 1.0 mL of ethyl acetate layer to new tube
12. Place tubes in a speed vac and evaporate the ethyl acetate to dryness (45 minutes)
13. Suspend dried extract in 20 uL of 100% ethanol (vortex)
14. Add 1.0 mL 1X sample dilution buffer from ELISA kit
15. Vortex vigorously *
16. Dilute 10X with sample dilution buffer
17. Add 100 uL to well of ELISA plate
18. Perform ELISA assay according to instruction booklet

*Centrifuge if there is any undissolved material

Assay Preparations

The solid 96-well plate and TMB solution are provided ready to use. The preparations of other assay reagents are detailed below.

Wash Buffer: Mix the solution, applying low heat until a clear colorless solution is obtained. Dilute the entire contents of the Wash Buffer Concentrate (25 mL) with 225 mL of deionized water to yield a final volume of 250 mL of 1 X Wash Buffer. This can then be refrigerated for the entire life of the kit.

HRP Conjugate: Dilute 1 vial of the BPA-HRP conjugate (0.012 mL) with 12.00 mL of 1 X HRP buffer. One vial makes enough conjugate for one plate. The conjugate must be used the same day and should not be stored for later use.

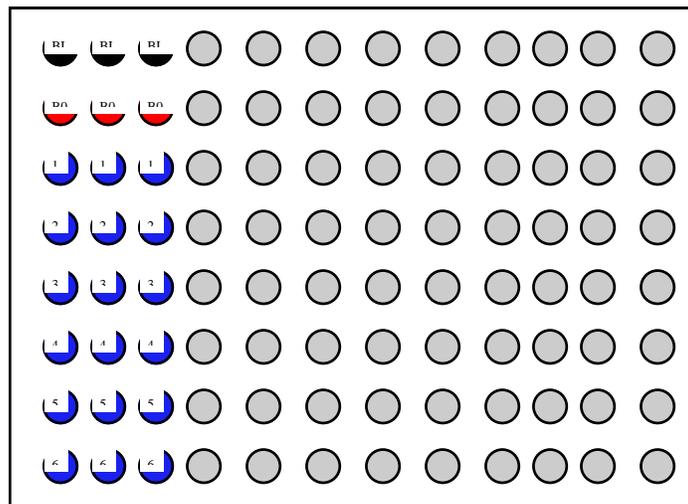
Standards: Label 5 microtubes as Standard 1 through Standard 5. Dilute the entire contents of Sample Dilution Stock buffer (25 mL) with 225 mL deionized water to yield a final volume of 250 mL of 1 X Sample Dilution Buffer. Add 0.9 mL of the Sample Dilution Buffer to the microtubes for Standards 1 to 5. Spin down the enclosed BPA standard vial (2 µL, filled with inert gas) and add 1.998 mL of Sample Dilution Buffer to obtain 2 mL of solution. Label this Standard 6. Add 0.1 mL of the Standard 6 to the microtube labeled Standard 5 and mix thoroughly. Next, add 0.1 mL of Standard 5 into the microtube labeled Standard 4 and mix thoroughly. Continue to serially dilute the standards using 1:10 dilutions for the remaining standards.

Samples: Samples can be directly diluted into the 1 X Sample Dilution Buffer if it is in solution. For extracted and dried samples, it is recommended to dissolve the dried-up samples with a minimal amount of ethanol of N, N-

dimethyl-formamide (DMF, 10 μ L to 20 μ L) and vortex well. Before ELISA assay, add 100 μ L of 1 X Sample Dilution Buffer to make the stock sample solution ready for quantification with ELISA. The stock sample solution can be further diluted to a proper range of concentration for ELISA test.

Performing the Assay

Plate Setup: Each plate must contain a minimum of three blank wells (B_L), three maximum binding wells (B_O), and a six point standard curve (S_1 - S_6). Each sample should be assayed in triplicate. A suggested plate format is shown below:



Standard Dilutions Table = B_L = B_O = S_1 — S_6 =Samples

Standards	Final Concentration (pg/mL)	Add Sample Dilution Buffer (mL)	Serial Dilutions Procedure
No. 6	1,000,000	1.998	2 μ L of stock solution.
No. 5	100,000	0.9	Add 0.1 mL of No. 6
No. 4	10,000	0.9	Add 0.1 mL of No. 5
No. 3	1000	0.9	Add 0.1 mL of No. 4
No. 2	100	0.9	Add 0.1 mL of No. 3
No. 1	10	0.9	Add 0.1 mL of No. 2

Assay Procedure

Step 1: Load 200 microliters of Sample Dilution Buffer into the blank (B_L) wells and 100 microliters of Sample Dilution Buffer into the maximum binding (B_O) wells.

Step 2: Load 100 microliters of each of the standards into the appropriate wells.

Step 3: Load 100 microliters of each of the samples into the appropriate wells.

Step 4: Load 100 microliters of the diluted BPA-HRP conjugate in the B_O wells, the standard wells, and the sample wells. Do NOT add HRP conjugate into the B_L wells.

Step 5: Incubate the plate at room temperature for two hours.

Step 6: Wash the plate three times with 400 microliters of the diluted Wash Buffer per well.

Step 7: After the last of the three wash cycles pat the plate dry onto some paper toweling.

Step 8: Add 200 microliters of the TMB substrate to all of the wells (including B_L wells).

Step 9: Incubate the plate at room temperature for 15-30 minutes.

Step 10: Add 50 micoliters of 2 N sulfuric acid to all of the wells.

Step 11: Read the plate at 450 nm.

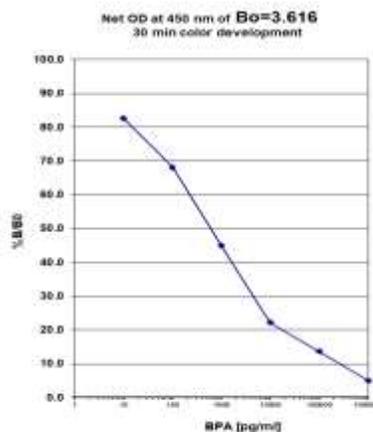
Calculating the Results

Most plate readers provide data reduction software that can be used to plot the standard curve and determine the sample concentrations. If your plate reader does not have this option, then a data reduction program can be used (4 parameter of log-log curve fit).

If you do not have these options, the results can be obtained manually as follows:

1. Average the absorbance readings from the blanks and subtract that value from each well of the plate to obtain the corrected readings. (Note: Some plate readers do this automatically. Consult the user manual of your plate reader.)
2. Average the corrected absorbance readings from the B_O wells. This is your maximum binding.
3. Calculate the %B/B_O for Standard 1 by averaging the corrected absorbance of the two S₁ wells, divide the average by the maximum binding, then multiply by 100. Repeat this formula for the remaining standards.
4. Plot the %B/B_O versus the concentration of BPA from the standards using semi-log paper.
5. Calculate the %B/B_O for the samples and determine the concentrations, utilizing the standard curve.
6. Multiply the concentrations obtained for each of the samples by their corresponding dilution factor.

Typical Results



The data shown here is an example of typical results obtained using the Detroit R & D BPA ELISA kit. These results are only a guideline, and should not be used to determine values from your samples. The user must run their own standard curve every time.

B_L wells = 0.060
 B_O wells = 3.676

Standard	Concentration	Net O.D.	%B/B _O
No. 1	10 pg/mL	3.616	82.6
No. 2	100 pg/mL	2.986	68.1
No. 3	1000 pg/mL	2.464	45.0
No. 4	10,000 pg/mL	1.628	22.2
No. 5	100,000 pg/mL	0.802	13.6
No. 6	1,000,000 pg/mL	0.492	5.0

Specificity of anti-BPA IgG

The specificity of the BPA ELISA was investigated using authentic BPA and a panel of bisphenols and related chemicals.

BPA	100%
BPF	<0.01%
BPS	<0.01%
Resveratrol	<0.01%

Troubleshooting

No color present in standard wells.

- The HRP conjugate was not added. Redo the assay and add the conjugate at the proper step.
- The HRP conjugate was not incubated for the proper time. Redo the assay and incubate for the proper time.

No color in any wells, including the TA wells.

- The TMB substrate was not added. Add substrate.
- The TMB substrate was not incubated for the proper time. Continue incubation until desired color is reached.

The color is faint.

- One or all of the incubation times were cut short. Redo the assay with the proper incubation times.
- The TMB substrate was not warmed up to room temperature. Redo the assay making sure all reagents are at room temperature.
- The lab is too cold. Be sure the lab temperature is between 21-27°C and redo the assay.

The background color is very high.

- The TMB substrate has been contaminated. Redo the assay with a fresh bottle of substrate.

Scattered O.D. obtained from the sample.

- Redo assay using an 8-channel pipetman making sure that 8 channels are equal volume while loading.

References

1. Zhao M, Zhou S, Yan J, Li L. Immunochemical analysis of endogenous and exogenous estrogens. *Current Pharmaceutical Analysis* (2007) 3, 25-38.
2. Völkel W, Colnot T, Csanády GA, Filser JG, Dekant W. Metabolism and kinetics of bisphenol a in humans at low doses following oral administration *Chem Res Toxicol* 15, 1281-1287 (2002).
3. Fourth National Report on human exposure to environmental chemicals. Centers` for Disease Control and Prevention (2009) www.cdc.gov/exposurereport.

Warranty

Detroit R&D, Inc., makes no warranty of any kind expressed, or implied, including, but not limited to the warranties of fitness for a particular purpose and merchantability.



Detroit R&D, Inc.

Metro Center For High Technology Bldg. (MCHT)
2727 Second Ave. Suite 4113
Detroit, MI 48201

Phone: 313.961.1606
Fax: 313.963.7130
E-mail: info@detroitrandd.com
www.DetroitRandD.com