ab175820 – Estrogen BPA Environmental ELISA Kit

Instructions for Use

A competitive immunoenzymatic assay for the quantitative measurement of BPA in urine, serum, plasma, cells and tissues.

This product is for research use only and is not intended for diagnostic use.

Version 6b Last Updated 22 November 2021

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1. BACKGROUND

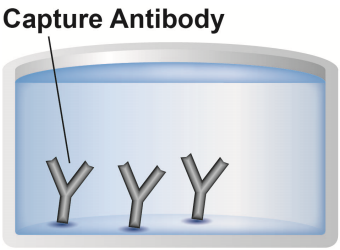
Abcam's Estrogen BPA Environmental ELISA Kit (ab175820) is designed for accurate quantitative measurement of Bisphenol A (BPA) in urine, serum, plasma, cells and tissues following proper isolation and purification.

BPA is a phenolic environmental estrogen which disrupts endocrine activity. In human, a BPA glucuronide was a primary metabolite of BPA. 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 creatinine. Urinary BPA levels were correlated with cardiovascular diseases and diabetes. 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 cans.

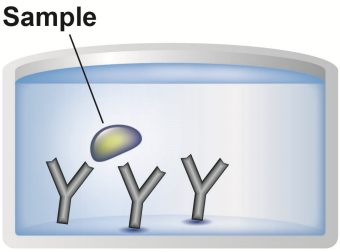
The free BPA level in urine or cell culture media can be measured using the BPA ELISA, without ethyl acetate extraction after 4-fold dilution of the sample.

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.

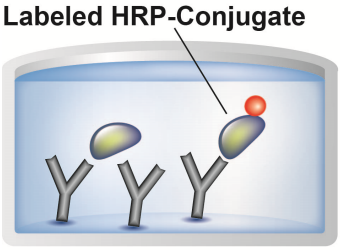
2. ASSAY SUMMARY



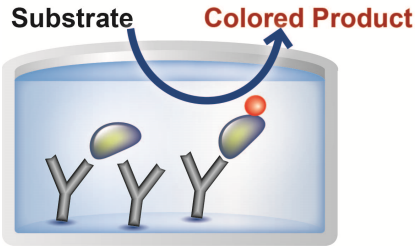
Prepare all reagents and samples as instructed.



Add standards and samples to each well used.



Add prepared HRP conjugate to each well and incubate at room temp.



Add TMB substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (before Preparation)	Storage Condition (After Preparation)
BPA ELISA Plate	96 Wells	-20°C	-20°C
BPA Standard	2 µL	-20°C	-20°C
BPA-HRP Conjugates	12 µL	-20°C	N/A
10X Sample Dilution Buffer	25 mL	-20°C	-20°C
HRP Buffer	15 mL	-20°C	-20°C
10X Wash Buffer Solution	25 mL	-20°C	-20°C
TMB Substrate	24 mL	-20°C	-20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

7. LIMITATIONS

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- Use only clean pipette tips, dispensers, and lab ware
- Do not interchange screw caps of reagent vials to avoid cross-contamination
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate, without splashing, accurately to the bottom of wells

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Equilibrate all reagents, samples and controls to room temperature (18-25°C) prior to use.

9.1 1X Wash Buffer

Mix the 10X Wash Buffer Solution with a stir bar, applying low, gentle heat until a clear colorless solution is obtained. Dilute the entire contents of the 10X Wash Buffer Solution (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.

9.2 1X HRP Conjugate

Dilute 1 vial of the BPA -HRP conjugate (12 µL) with 12 mL of 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.

9.3 1X Sample Dilution Buffer

Prepare 1X Sample Dilution Buffer by adding 25 mL of 10X Sample Dilution Buffer to 225 mL of dH_20. Mix gently and thoroughly. Store reagents at -20°C.

10. STANDARD PREPARATION

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of positive controls for every use.

10.1 Label 5 microtubes as Standard # 2 - 6.

10.2 Add 900 µL of the 1X Sample Dilution Buffer to the microtubes for Standards # 2 to 6.

10.3 Prepare a 1 µg/mL **Standard #1** by first spinning down the enclosed BPA standard vial (2 µL, filled with inert gas) and then adding 1.998 mL of 1X Sample Dilution Buffer to obtain 2 mL of solution.

10.4 Prepare **Standard #2** by adding 100 µL of the Standard #1 to the microtube labeled **Standard #2**. Mix thoroughly and gently.

10.5 Prepare **Standard #3** by adding 100 µL of the Standard #2 to the microtube labeled **Standard #3**. Mix thoroughly and gently.

10.6 Using the table below as a guide, repeat for tubes #4 through #6.

10.7 Standard B_o contains no protein and is blank control

Standard #	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Step 10.3				1,000,000
2	Standard #1	100	900	1,000,000	100,000
3	Standard #2	100	900	100,000	10,000
4	Standard #3	100	900	10,000	1,000
5	Standard #4	100	900	1,000	100
6	Standard #5	100	900	100	10
B_o	None		900	-	

11. SAMPLE COLLECTION AND STORAGE

There are different protocols for isolating and purifying BPA depending on the medium in which it is in. Listed below are the different protocols for sample preparation. For optimal results follow the appropriate protocol based on the biological sample present.

11.1 **BPA measurement in urine**

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

11.1.2 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).

11.2 **BPA measurement in water**

11.2.1 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.

11.3 **BPA measurement in cells**

11.3.1 Collect and homogenize and/or sonicate the cells.

11.3.2 Acidify the whole homogenized cells with acetic acid to a pH of approximately 3-4. Measure using standard pH paper.

11.3.3 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.

11.3.4 Evaporate the pooled ethyl acetate from the extractions until all has dried up under argon or nitrogen gas.

11.3.5 Add 10 µL to 20 µL ethanol, or N, N-dimethyl-formamide (DMF), to reconstitute the dried-up residue from above step # 11.2.4. 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.)

11.3.6 Perform the ELISA for BPA (according to the instructions of the manufacturer).

11.3 BPA measurement in tissues

11.3.1 Homogenize 1 g of tissue, 4 mL of H_2O.

11.3.2 Acidify the homogenate by adding 8 µL of acetic acid to each homogenate.

11.3.3 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.

11.3.4 Dry the organic phase with argon or nitrogen gas.

11.3.5 Dissolve the dried residue from above step with ethanol or DMF. (Add approximately 20 µL of

ethanol or DMF to reconstitute the dried-up residue.)

11.3.6 Dilute further with 1X Sample Dilution Buffer: Add approximately 500 µL of 1x Sample Dilution Buffer and centrifuge at 10,000 rpm for five minutes at room temperature. The supernatant will be used for ELISA.

11.3.7 Perform the ELISA for BPA (according to the instructions of the manufacturer).

11.4 BPA measurement in plasma or serum

11.4.1 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:

- 11.4.1.1 Upper organic phase – ethyl acetate phase (lipoproteins)
- 11.4.1.2 Interphase – proteins
- 11.4.1.3 Lower phase – aqueous phase

11.4.2 Collect the upper organic phase (a) and set aside.

11.4.3 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.

11.4.4 Evaporation of pooled organic phase: There should be approximately 3 mL of the ethyl acetate phase (a). Dry the pooled organic phase in a Speedvac to get the extracted sediment (b).

11.4.5 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, and then add 130 µL of 1x Sample Dilution Buffer.

11.4.6 For the competitive BPA ELISA, the above 150 µL sample needs to be further diluted: When

calculating the concentration, consider any dilution factors.

11.4.7 Perform the ELISA for BPA (according to the instructions of the manufacturer).

11.5 BPA measurement in canned food

11.5.1 Strain liquid portion from any solids

11.5.2 Measure volume of liquid (or weigh)

11.5.3 Transfer liquid to centrifuge tube and spin at 1000g for 4 minutes at 4°C to remove any remaining solids.

11.5.4 Save supernatant

11.5.5 Dilute supernatant 4-fold (more or less dilution may be required) and add 100 uL of sample to well of

11.5.6 ELISA plate.

11.5.7 Perform ELISA as described in Instruction Booklet provided with kit

11.6 BPA measurement in animal chow

11.6.1 Weigh out 6.5 g of each lab chow

11.6.2 Pulverize lab chow with porcelain mortar and pestle

11.6.3 Placed in 50 mL tubes and added 10 mL HPLC grade water to powdered lab chow

11.6.4 Vortex vigorously for 30 seconds

11.6.5 Place on platform rocker and shake for 1 hour

11.6.6 Sonicate for 20 seconds at 80% full power

11.6.7 Vortex vigorously

11.6.8 Remove 2.0 mL to new tube

11.6.9 Add 3.0 mL of ethyl acetate and vortex

11.6.10 Centrifuge at 3000 g for 5 minutes to separate layers

11.6.11 Remove 1.0 mL of ethyl acetate layer to new tube

11.6.12 Place tubes in a speed vac and evaporate the ethyl acetate to dryness (45 minutes)

11.6.13 Suspend dried extract in 20 uL of 100% ethanol (vortex)

11.6.14 Add 1.0 mL 1X sample dilution buffer from ELISA kit

11.6.15 Vortex vigorously *

11.6.16 Dilute 10X with sample dilution buffer

11.6.17 Add 100 uL to well of ELISA plate

11.6.18 Perform ELISA assay according to instru

*centrifuge if there is any undissolved material

12. PLATE PREPARATION

- The 96 well plate included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at -20°C.
- For each assay performed, a minimum of 2 wells must be used as a blank, omitting sample and conjugate from well addition. Another 2 wells must be used for a maximum binding control.
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates).

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use**
- **Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described**
- **If performing the test on an automatic ELISA system we recommend increasing the washing steps from three to five and the volume of 1X Wash Buffer from 300 µL to 350 µL to avoid washing effects**
- **Assay all standards, controls and samples in duplicate**

13.1 Add 200 µL of 1X Sample Dilution Buffer into the blank wells and 100 µL of 1X Sample Dilution Buffer into maximum binding control wells.

13.2 Add 100 µL of each of the standards or samples into the appropriate wells.

13.3 Add 100 µL of each of the samples into the appropriate wells.

13.4 Add 100 µL of the 1X-HRP conjugate in the all wells except the blank control wells.

13.5 Incubate the plate at room temperature for two hours.

13.6 Wash the plate three times with 400 µL of 1X Wash Buffer per well.

13.7 After the last of the three wash cycles pat the inverted plate dry onto some paper towels.

13.8 Add 200 µL of the TMB substrate to all of the wells.

13.9 Incubate the plate at room temperature for 15-30 minutes.

13.10 Add 50 µL of 2 N sulfuric acid to all of the wells.

13.11 Read the plate at 450 nm.

14. CALCULATIONS

If data redaction software is not available on your plate reader then the results can be obtained manually as follows:

14.1 Average the absorbance (Abs) readings from the blank wells 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.)

14.2 Average the corrected absorbance readings from the maximum binding control wells. This is your maximum binding.

14.3 Calculate the % Abs for Standard 1 by averaging the corrected absorbance of the two wells; divide the average by the Maximum Binding Control well average absorbance, then multiply by 100. Repeat this formula for the remaining standards.

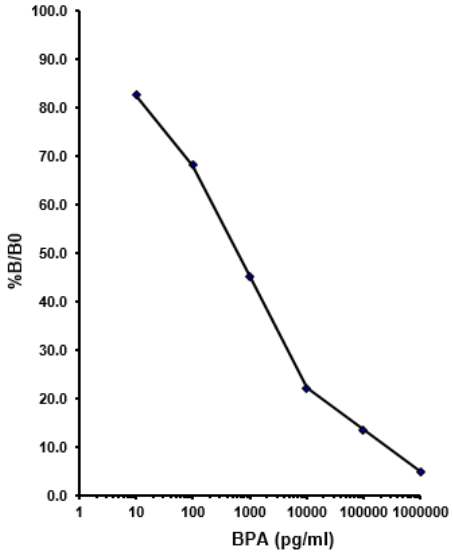
14.4 Plot the % Abs versus the concentration of BPA from the standards using semi-log paper.

14.5 Calculate the % Abs for the samples and determine the concentrations, utilizing the standard curve.

14.6 Multiply the concentrations obtained for each of the samples by their corresponding dilution factor.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed. The data shown here is an example of typical results obtained using the Abcam's BPA ELISA kit. These results are only a guideline, and should not be used to determine values from your samples.



Conc. (pg/mL)	% (B/B_0)
1	82.6
10	68.1
100	45.0
1,000	22.2
10,000	13.6
100,000	5.0

16. ASSAY SPECIFICITY

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

Chemicals	Reactivity
BPA	100.00%
BPF	<0.01%
BPS	<0.01%
Resveratrol	<0.01%

17. TROUBLESHOOTING

Problem	Cause	Solution
Low signal	Incubation time to short	Try overnight incubation at 4 °C
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes & ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)

Problem	Cause	Solution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

18. NOTES

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)