

ab175820 – Estrogen BPA Environmental ELISA Kit

Instructions for Use

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

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

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1. BACKGROUND

Abcam's Estrogen BPA Environmental competitive *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for of Estrogen BPA Environmental in urine, serum, plasma, cells, and tissues following proper isolation and purification.

Estrogen BPA Environmental is a phenolic environmental estrogen¹ which disrupts endocrine activity. In human, an Estrogen BPA Environmental glucuronide was a primary metabolite of BPA. In a recent study, the age group with highest Estrogen BPA Environmental exposure was 6-11 years old with a mean total (free + glucuronidated) Estrogen BPA Environmental level of 4.33 ng/g of creatinine. Urinary Estrogen BPA Environmental 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 Estrogen BPA Environmental level 12-fold due to Estrogen BPA Environmental -containing epoxy resin lining of the cans.

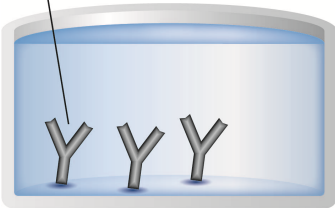
The free Estrogen BPA Environmental level in urine or cell culture media can be measured using the Estrogen BPA Environmental ELISA, without ethyl acetate extraction after 4-fold dilution of the sample. The glucuronidated Estrogen BPA Environmental level can also be measured without extraction after beta-glucuronidase treatment using our specific protocol.

INTRODUCTION

This competitive ELISA kit, based on competition between the Estrogen BPA Environmental epitope and Estrogen BPA Environmental-HRP conjugate for a limited number of binding sites available from the anti- Estrogen BPA Environmental 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 Estrogen BPA Environmental is variable, based on the concentration of the sample or standard. Thus the amount of the Estrogen BPA Environmental conjugate which is able to bind to each of the wells is inversely proportional to the concentration of Estrogen BPA Environmental 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.

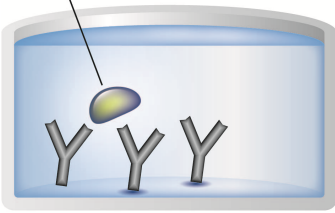
2. ASSAY SUMMARY

Capture Antibody



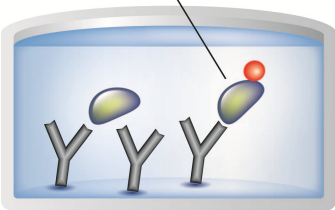
Prepare all reagents and samples as instructed.

Sample



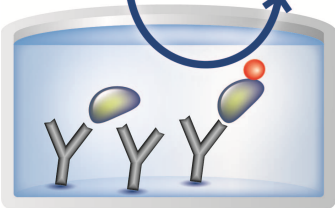
Add standards and samples to each well used.

Labeled HRP-Conjugate



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

Substrate **Colored Product**



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

Store kit at 2-8°C or -20°C immediately upon receipt.

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 (After Preparation)
BPA ELISA Plate	96 Wells	-20°C
BPA Standard	2 µL	-20°C
BPA-HRP Conjugates	12 µL	-20°C
10X Sample Dilution Buffer	25 mL	-20°C
HRP Buffer	15 mL	-20°C
10X Wash Buffer Solution	25 mL	-20°C
TMB Substrate	24 mL	-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 Estrogen BPA Environmental -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 dH₂O. Mix gently and thoroughly.

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 $\mu\text{g}/\text{mL}$ **Standard #1** by first spinning down the enclosed Estrogen BPA Environmental 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 B₀ 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 ₀	None		900	-	



11. SAMPLE COLLECTION AND STORAGE

There are different protocols for isolating and purifying Estrogen BPA Environmental 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 **Estrogen BPA Environmental measurement in urine**

- 11.1.1 Make 8.0 mL of a 1.0 M citrate buffer solution, pH 5.5
- 11.1.2 Dissolve the 8 mg of beta-glucuronidase enzyme provided with the kit in 8.0 mL of the 1M citrate buffer.
- 11.1.3 Centrifuge the urine sample to remove any solids
- 11.1.4 Dilute 1.0 mL of human urine 4-fold with 1X Sample Dilution Buffer. (You must dilute the 10X Sample Dilution Buffer provided in the kit).
- 11.1.5 Divide the diluted urine sample into two equal aliquots of 2.0 mL
- 11.1.6 Add 0.5 mL of the beta-glucuronidase enzyme solution you prepared ahead of time to both of the diluted 2.0 mL urine aliquots.

- 11.1.7 To one of the aliquots, quickly add 2.0 mL of ethyl acetate, vortex vigorously and centrifuge for 3 minutes at > 5,000 g to separate the aqueous and organic layers.
- 11.1.8 Remove and save the ethyl acetate layer in a clean tube and place on ice. This is the zero time point sample.
- 11.1.9 Incubate the other 2.0 mL aliquot at 37°C for 3 hours (A shaking water bath is recommended). This is the 3 hour time point sample.
- 11.1.10 After the three hour incubation, extract with ethyl acetate as you did with the zero time point sample.
- 11.1.11 Evaporate the ethyl acetate using a speed vac or a gentle stream of nitrogen or argon.
- 11.1.12 Resuspend the dried extract with 20 µL of ethanol. Vortex sample to ensure the extract is completely dissolved.
- 11.1.13 Add 1.0 mL of the 1X Sample Dilution Buffer to the tube.
- 11.1.14 Add 100 µL to the sample wells of the ELISA plate. If necessary, the sample can be diluted to ensure that the absorbance reading of the well falls within the limits of the standard curve.
- 11.1.15 Follow the instructions in the Estrogen BPA Environmental instruction booklet to perform the ELISA.
- 11.1.16 Subtract the value obtained with the zero time point from the 3 hour time point for a measurement of the Estrogen BPA Environmental level in the urine sample. Make sure to include all dilution factors in your calculations.

11.2 Estrogen BPA Environmental measurement in cells

- 11.2.1 Collect and homogenize and/or sonicate the cells.
- 11.2.2 Acidify the whole homogenized cells with acetic acid to a pH of approximately 3-4. Measure using standard pH paper.
- 11.2.3 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.
- 11.2.4 Evaporate the pooled ethyl acetate from the extractions until all has dried up under argon or nitrogen gas.
- 11.2.5 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.)
- 11.2.6 Perform the ELISA for Estrogen BPA Environmental (according to the instructions of the manufacturer).

11.3 Estrogen BPA Environmental 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 Estrogen BPA Environmental (according to the instructions of the manufacturer).

11.4 Estrogen BPA Environmental 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 Estrogen BPA Environmental 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 Estrogen BPA Environmental (according to the instructions of the manufacturer).

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 4°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 the 1X-HRP conjugate in the all wells except the blank control wells.
- 13.4 Incubate the plate at room temperature for two hours.
- 13.5 Wash the plate three times with 400 μL of 1X Wash Buffer per well.
- 13.6 After the last of the three wash cycles pat the inverted plate dry onto some paper towels.
- 13.7 Add 200 μL of the TMB substrate to all of the wells.
- 13.8 Incubate the plate at room temperature for 15-30 minutes.
- 13.9 Add 50 μL of 2 N sulfuric acid to all of the wells.
- 13.10 Read the plate at 450 nm.

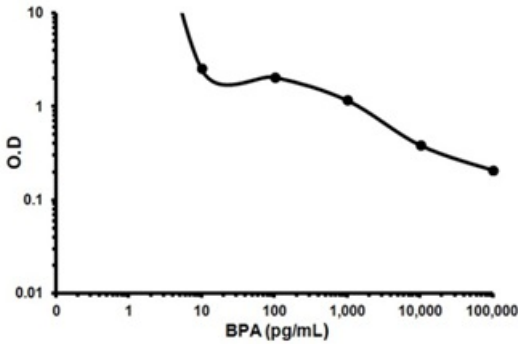
14. CALCULATIONS

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 Estrogen BPA Environmental 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 Estrogen BPA Environmental ELISA kit. These results are only a guideline, and should not be used to determine values from your samples.



Conc. (pg/mL)	O.D	% (Abs Blank / Abs Sample)
1	2.834	98.7
10	2.555	89.0
100	2.077	72.3
1,000	1.172	40.8
10,000	0.388	13.5
100,000	0.209	7.3

Blank well mean abs = 0.052

Maximum Control Well mean abs = 2.870

16. ASSAY SPECIFICITY

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

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

SENSITIVITY-

The calculated minimal detectable (MDD) dose is 3 pg/mL. The MDD was determined by calculating the mean of zero standard replicates.

17. TROUBLESHOOTING

Problem	Cause	Solution
Low signal	Incubation time too 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)

RESOURCES

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

UK, EU and ROW

Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria

Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France

Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany

Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain

Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland

Email: technical@abcam.com

Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America

Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada

Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific

Email: hk.technical@abcam.com | Tel: 108008523689 (中國聯通)

Japan

Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp