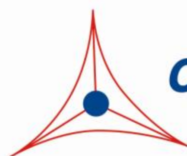
Product Manual

Bisphenol A (BPA) Competitive ELISA Kit

Catalog Number

AKR-5134	96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction
Bisphenol A (BPA) is a synthetic compound containing two hydroxyphenyl groups and belonging to the group of diphenylmethane derivatives. BPA is a building block in the synthesis of polymer plastics, including some polycarbonate resins, epoxy resins, as well as polysulfones. BPA-based plastic is a strong transparent material and can be used for a wide range of consumer goods: plastic water bottles, food storage containers, baby bottles, sports equipment, compact discs, and digital video discs. Epoxy resins created from BPA are used to coat water pipes, to coat the interior of food cans and to make thermal paper commonly used in sales receipts.

BPA has estrogen-mimicking properties. Although its effect is not robust, the routine use of BPA-containing products has heightened fears. As a result of government studies, some large businesses have decided to withdraw their BPA-containing products. BPA binds both of the nuclear estrogen receptors (ERs), ERα and ERβ, although it should be noted that the affinity is 1-2000 fold less potent than estradiol. BPA can both agonize and antagonize the effects of estrogen, suggesting that BPA is a modulator or partial agonist of the ER. At high concentrations, BPA also serves as an antagonist of the androgen receptor (AR). In addition to receptor binding, BPA has been shown to influence Leydig cell steroidogenesis, including altering the expression of 17α-hydroxylase/17,20 lyase as well as aromatase. In 1997, negative effects of BPA at low doses were first observed in laboratory animals. More recent studies found possible developmental health issues caused by exposure to BPA during pregnancy.

Cell Biolabs' Bisphenol A (BPA) Competitive ELISA Kit provides a convenient method for the detection of total BPA in extracts from cells, tissue, feces, serum, plasma, urine, or foods. The total content of BPA in unknown extracted samples is determined by comparison with a BPA standard curve. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown protein samples. The kit has a detection sensitivity limit of 25 nM BPA.

Assay Principle
First, BPA conjugate is coated on an ELISA plate. The unknown extracted BPA samples or BPA standards are then added to the BPA conjugate preadsorbed ELISA plate. After a brief incubation, an anti-BPA monoclonal antibody is added, followed by an HRP conjugated secondary antibody. The total content of BPA in unknown extracted samples is determined by comparison with a BPA standard curve.

Related Products
1. AKR-360: Histamine Assay Kit
2. STA-301: OxiSelect™ BPDE Protein Adduct ELISA Kit
3. STA-357: OxiSelect™ BPDE DNA Adduct ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. 96-well Protein Binding Plate (Part No. 231001): One strip well 96-well plate.
2. Anti-BPA Antibody (500X) (Part No. 51341C): One 10 µL vial of anti-BPA Antibody.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 230003): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. BPA Standard (Part No. 51342C): One 50 µL vial of 150 µM BPA.
2. BPA Conjugate (500X) (Part No. 51343C): One 25 µL vial.
3. 100X Conjugate Diluent (Part No. 281603): One 300 µL vial.

Materials Not Supplied

1. 1X PBS
2. Bovine Serum Albumin (BSA)
3. Acetonitrile
4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
6. Multichannel micropipette reservoir
7. Microplate reader capable of reading at 450 nm (620 nm as optional reference wavelength)
8. Probe sonicator
9. 20 mL Glass Vials

Storage

Upon receipt, store Anti-BPA Antibody (500X), Secondary Antibody HRP Conjugate (1000X), BPA Standard, BPA Conjugate, and 100X Conjugate Diluent at -20ºC. Store all the remaining components at 4°C.

Preparation of Reagents

- BPA Conjugate Coated Plate:

  Note: The BPA Conjugate coated wells are not stable and should be used within 24 hrs after coating. Only coat the number of wells to be used immediately.

1. Immediately before use, prepare 1X Conjugate Diluent by diluting the 100X Conjugate Diluent in 1X PBS. Example: Add 50 µL to 4.95 mL of 1X PBS.
2. Immediately before use, prepare 1X BPA Conjugate by diluting the 500X BPA Conjugate in 1X Conjugate Diluent. Example: Add 10 µL of 500X BPA Conjugate to 4.99 mL of 1X Conjugate Diluent.
3. Add 100 µL of the 1X BPA Conjugate to each well to be tested and incubate overnight at 4ºC. Remove the BPA Conjugate coating solution and wash twice with 1X PBS. Blot plate on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1 hr at room temperature on an orbital shaker. Transfer the plate to 4ºC and remove the Assay Diluent **immediately before use.**

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-BPA Antibody and Secondary Antibody: Immediately before use, dilute the Anti-BPA antibody 1:500 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Prepare a dilution series of BPA standards in the concentration range of 0 to 1500 nM by diluting the BPA Standard in Assay Diluent (Table 1).

Standard Tubes	150 µM BPA Standard (µL)	Assay Diluent (µL)	BPA (nM)
1	5	495	1500
2	200 of Tube #1	200	750
3	200 of Tube #2	200	375
4	200 of Tube #3	200	188
5	200 of Tube #4	200	94
6	200 of Tube #5	200	47
7	200 of Tube #6	200	23
8	0	200	0

Table 1. Preparation of BPA Standards

Preparation of Samples

Note: Once samples have been initially prepared, bound BPA must be extracted from proteins by the acetonitrile protocol described below.

- Serum: Avoid hemolyzed and lipemic blood samples. Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Aliquot samples for testing and store at -80ºC until ready to perform acetonitrile extraction described below.
- Plasma: Avoid hemolyzed and lipemic blood samples. Collect blood with heparin or citrate and centrifuge at 2000 x g and 4ºC for 10 minutes. Remove the plasma layer and store on ice. Avoid

disturbing the white buffy layer. Aliquot samples for testing and store at -80ºC until ready to perform acetonitrile extraction described below.

- Cells, tissues, or feces: Homogenize 50-200 mg of the cell pellet, tissue, or feces in 0.5-2 mL of ice cold PBS using a mortar and pestle or by dounce homogenization. Incubate the homogenate at 4°C for 20 minutes. Transfer the homogenate to a centrifuge tube and centrifuge at 12000 x g for 20 minutes. Recover the supernatant and transfer to a fresh tube. Store resuspended sample at -20°C or colder until ready to perform acetonitrile extraction described below.
- Food samples: Homogenize 1-5 grams using a mortar and pestle or by dounce homogenization. Store homogenized sample at -20°C or colder until ready to perform acetonitrile extraction described below.

Acetonitrile Extraction of Bound BPA

1. Add 1-5 mLs or grams of unknown samples to a 50 mL conical tube.
2. Add 6 volumes of acetonitrile per mL or gram of sample.
3. Sonicate or homogenize in an ice bath.
4. Transfer the homogenate to a centrifuge tube and centrifuge the homogenate at 10000 x g for 10 minutes at 4°C. Recover the supernatant and transfer to a glass vial.
5. Incubate the open vial at 80°C for 4 hours to overnight in an oven or heat block to dry.
6. Cool the vial to room temperature and resuspend the dried extract in 0.2-5 mL of distilled water.

   Note: Choose an appropriate volume based on the expected BPA levels of your sample. If low levels of BPA are expected, choose a final volume that is lower than the starting volume. Note the difference in final volume from the starting volume of your unknown sample.

7. Cap the tube and vortex well to resuspend the dried material.
8. Store at 4°C. Dilute as necessary into assay diluent or 1X PBS containing 0.1% BSA.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each BPA sample including unknown and standard should be assayed in duplicate.
2. Add 50 µL of unknown sample or BPA standard to the wells of the BPA Conjugate coated plate. Incubate at room temperature for 10 minutes on an orbital shaker.
3. Add 50 µL of the diluted anti-BPA antibody to each well, incubate at room temperature for 1 hour on an orbital shaker.

4. Wash 3 times with 250 µL of 1X Wash Buffer with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Secondary Antibody-HRP Conjugate to all wells and incubate for 1 hour at room temperature on an orbital shaker. Wash the strip wells 3 times according to step 4 above.
6. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well. Incubate at room temperature for 2-20 minutes on an orbital shaker.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
7. Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time).
8. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical BPA Competitive ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

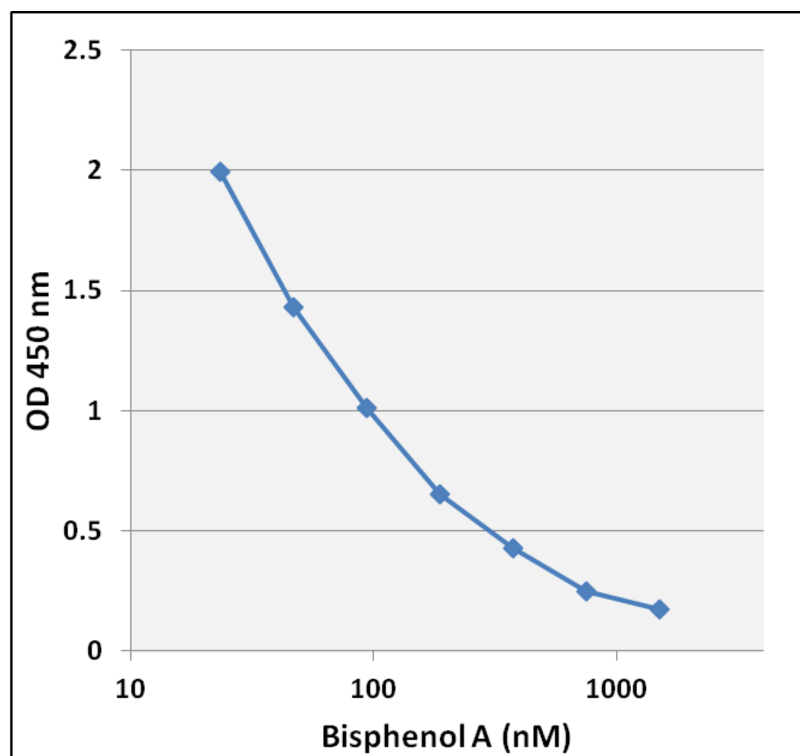


Figure 1: BPA Standard Curve.

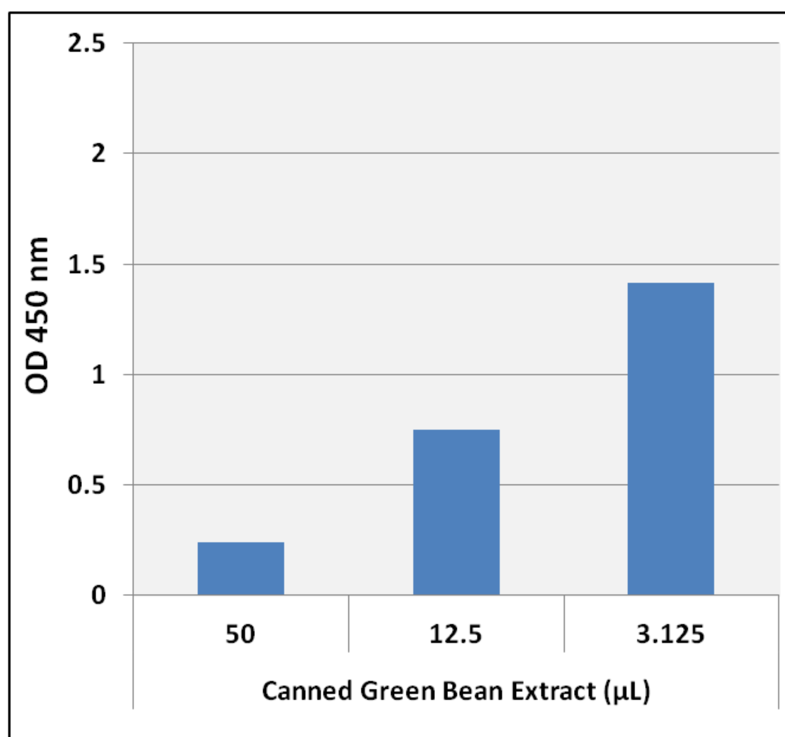


Figure 2: Detection of BPA in canned green beans. Canned Fresh Cut Green Beans (Del Monte) were processed using the above Acetonitrile Extraction protocol. The BPA levels were measured using the Bisphenol A Competitive ELISA Kit.

References

1. Pivnenko Km Pedersen GA, Eriksson E, and Astrup T F (2015). *Waste Management*. **44**: 39–47.
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5. Cao XL, Perez-Locas C, Dufresne G, Clement G, Popovic S, Beraldin F, Dabeka RW, and Feeley M. (2011) *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*. **28**:791-798.

Warranty

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