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User's Manual

Tetrabromobisphenol A ELISA Kit

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The Tetrabromobisphenol A ELISA Kit is an immunoassay for detection of Tetrabromobisphenol A(TBBPA).

General Description

Tetrabromobisphenol A (TBBPA) is a brominated flame retardant. TBBPA is mainly used as a reactive component of polymers, meaning that it is incorporated into the polymer backbone. It is used to prepare fire-resistant polycarbonates by replacing some bisphenol A. A lower grade of TBBPA is used to prepare epoxy resins, used in printed circuit boards. Some studies suggest that TBBPA may be an endocrine disruptor and immunotoxicant. As an endocrine disruptor, TBBPA may interfere with both estrogens and androgens. Further, TBBPA structurally mimics the thyroid hormone thyroxin (T4) and can bind more strongly to the transport protein transthyretin than T4 does, likely interfering with normal T4 activity.

Principles of Testing

1. Competitive Reaction

The test is based on the recognition of TBBPA by specific monoclonal antibodies. TBBPA present in the sample and a TBBPA-enzyme conjugate are premixed and added into each well of a microplate, and allowed to compete for limited number of binding sites of specific antibodies immobilized on the surface of the wells. When the TBBPA concentration is higher relative to the enzyme conjugate, the TBBPA will predominantly bind the antibody and vice versa.

2. Chromogenic Reaction

Unbound TBBPA and excess TBBPA-enzyme conjugates are washed out. The presence of TBBPA is detected by adding a chromogenic substrate. The enzyme-labeled TBBPA bound to the TBBPA antibody in the plate, catalyzes the conversion of the substrate to a colored product. After an incubation period, the reaction is stopped by the addition of a diluted acid. The higher the TBBPA concentration in a sample, for example, leads to less antigen-enzyme conjugate bound to the antibody binding sites in a microplate well, generating a lighter color, i.e. lower absorbance.

3. Quantitative Analysis

The standard curve, a dose-response curve obtained from known concentrations of TBBPA standards, is determined from the absorbance at 450nm. The TBBPA concentration in each sample is accurately calculated by interpolation using the absorbance intensity obtained from the standard curve.

Reagents And Materials Provided

1. MoAb-Coated Microplate 96 Wells
2. TBBPA Standard
3. Antigen-enzyme Conjugate
4. Buffer Solution
5. Wash Solution

6. Color Solution
7. Stop Solution
8. Plate Cover
9. Instruction Booklet

Materials Required But Not Supplied

1. Glass disposable test tubes. Be sure to use disposable tubes to avoid BPA adsorption.
2. Micropipettes (20µL - 200µL and 100µL - 1000µL) and tips
3. Multichannel pipettes and tips
4. Microplate reader (450nm wavelength)
5. Stop watch
6. Methanol (HPLC grade)
7. Glass fiber filters and filtering equipment

Storage

Store all reagents at 2-8°C. Do not freeze. Reagents may be used until the expiration date on the box.

Specimen Collection And Preparation

Filter raw water samples through the specified glass fiber filter (1µm pore diameter).

Assay Procedure

1. Reconstitute a bottle of antigen-enzyme conjugate powder with buffer solution to prepare antigen-enzyme conjugate solution.
2. Transfer 100µL of conjugate solution, and then transfer 100µL of TBBPA standard or 100µL of sample, prepared as 10 % (v/v) methanol solution, into each well of the uncoated microplate and mix by filling the tip and expelling the contents with a pipette.
3. Dispense 100µL aliquots of the mixture, prepared in the above Section 2, into each coated well of the microplate. Tap the plate lightly to make the liquid level horizontal. Incubate the microplate for 60 minutes at room temperature (18-25°C).
4. Rinse each microplate well with approximately 300µL of the wash solution and repeat the step twice more. Then, firmly tap out the plate to remove solution from the microplate. Blotting the plate against a paper towel, a clean cloth or a lint-free towel is helpful.
5. Dispense 100µL of the color solution into each microplate well and incubate the microplate for 30 minutes at room temperature (18-25°C). Then, add 100µL of Stop Solution to terminate the reaction.
6. Read the absorbance at 450nm for each standard solution and samples with a plate reader.