Bisphenol A (BPA) Assay Kit - IBL

INTRODUCTION

Bisphenol A (BPA), principal raw material for epoxy resins and polycarbonate, is suspected as one of the endocrine disrupting chemicals. BPA is a chemical building block for making polycarbonate plastic used for food containers and feeding bottles, which touches mouths directly, and is also used in production of epoxy resins for food and beverage can-linings. In Japan, more than 4.8 million tons of BPA are produced each year and the water survey report from ministry of the environment says that there is 0.11μg/L of BPA in rivers. So, we are anxious about the adversely impact for aquatic organisms and ecosystems. Vom Saal reported that in pregnant mice dosed at 2 μg per kg of body weight with a level of 1/25 of the threshold limit value per day, the prostate of the male offspring was enlarged. This product is made for research use only by measuring BPA in serum of plasma; it is based on an ELISA using anti-rabbit IgG antibody coated solid-phase method.

PRINCIPLE

This kit is based on a competitive ELISA using anti-rabbit IgG antibody solid-phase method. BPA standard or sample is taken on a anti-rabbit IgG antibody coated microplate and enzyme-labeled BPA and anti-BPA serum are added to cause a reaction. After the reaction is completed, the plate is washed and the enzyme reaction is allowed to proceed with addition of a substrate. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The absorbance is then measured by using a plate reader. A standard curve is draw, and the BPA concentration in the reaction is allowed to proceed with addition of a substrate.

OPERATION MANUAL

1. Materials needed but not supplied
   - Plate reader (450nm)
   - Micropipette and tip
   - Graduated cylinder and beaker
   - Deionized water
   - Paper towel
   - Tap the plate for mixing, and then read the plate at 450nm

2. Preparation
   Preparation of wash buffer
   - "Wash buffer Conc." is a concentrated (450X) buffer. Adjust the temperature of "8, Washing buffer Conc." to room temperature and then, mix it gently and completely before use. Dilute 50 mL of "8, Wash buffer Conc." with 1.950 mL of deionized water and mix it. This is the wash buffer for use.

3. Measurement procedure
   All reagents shall be brought to room temperature before use. Then mix it gently and completely before use. Make sure of no change in quality of the reagents.

4. Enzyme reaction
   After the reaction is completed, the plate is washed and the enzyme reaction is allowed to proceed with addition of a substrate. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The absorbance is then measured by using a plate reader. A standard curve is drawn, and the BPA concentration in the sample is obtained.

5. Measurement
   After the reaction is completed, the plate is washed and the enzyme reaction is allowed to proceed with addition of a substrate. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The absorbance is then measured by using a plate reader. A standard curve is drawn, and the BPA concentration in the sample is obtained.

6. Enzyme reaction
   After the reaction is completed, the plate is washed and the enzyme reaction is allowed to proceed with addition of a substrate. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The absorbance is then measured by using a plate reader. A standard curve is drawn, and the BPA concentration in the sample is obtained.

7. Enzyme reaction
   After the reaction is completed, the plate is washed and the enzyme reaction is allowed to proceed with addition of a substrate. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The absorbance is then measured by using a plate reader. A standard curve is drawn, and the BPA concentration in the sample is obtained.

8. Enzyme reaction
   After the reaction is completed, the plate is washed and the enzyme reaction is allowed to proceed with addition of a substrate. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The absorbance is then measured by using a plate reader. A standard curve is drawn, and the BPA concentration in the sample is obtained.

9. Enzyme reaction
   After the reaction is completed, the plate is washed and the enzyme reaction is allowed to proceed with addition of a substrate. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The absorbance is then measured by using a plate reader. A standard curve is drawn, and the BPA concentration in the sample is obtained.

10. Enzyme reaction
    After the reaction is completed, the plate is washed and the enzyme reaction is allowed to proceed with addition of a substrate. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The absorbance is then measured by using a plate reader. A standard curve is drawn, and the BPA concentration in the sample is obtained.

11. Enzyme reaction
    After the reaction is completed, the plate is washed and the enzyme reaction is allowed to proceed with addition of a substrate. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The absorbance is then measured by using a plate reader. A standard curve is drawn, and the BPA concentration in the sample is obtained.

12. Enzyme reaction
    After the reaction is completed, the plate is washed and the enzyme reaction is allowed to proceed with addition of a substrate. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The absorbance is then measured by using a plate reader. A standard curve is drawn, and the BPA concentration in the sample is obtained.

13. Enzyme reaction
    After the reaction is completed, the plate is washed and the enzyme reaction is allowed to proceed with addition of a substrate. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The absorbance is then measured by using a plate reader. A standard curve is drawn, and the BPA concentration in the sample is obtained.

14. Enzyme reaction
    After the reaction is completed, the plate is washed and the enzyme reaction is allowed to proceed with addition of a substrate. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The absorbance is then measured by using a plate reader. A standard curve is drawn, and the BPA concentration in the sample is obtained.

15. Enzyme reaction
    After the reaction is completed, the plate is washed and the enzyme reaction is allowed to proceed with addition of a substrate. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The absorbance is then measured by using a plate reader. A standard curve is drawn, and the BPA concentration in the sample is obtained.
**PERFORMANCE CHARACTERISTICS**

1. **Sensitivity**
   1) The absorbance of BPA at 0 ng/mL ($B_0$) is 1.5 or more.
   2) The absorbance ratio of standard 100 ng/mL ($B_{100}$) to standard 0 ng/mL ($B_0$) is less than 20%.

2. **Intra Assay**

<table>
<thead>
<tr>
<th>Measurement Value (ng/mL)</th>
<th>SD value</th>
<th>CV value (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.32</td>
<td>0.19</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>4.49</td>
<td>0.38</td>
<td>8.5</td>
<td>8</td>
</tr>
<tr>
<td>14.73</td>
<td>1.03</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>31.15</td>
<td>1.72</td>
<td>5.5</td>
<td>8</td>
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3. **Specificity**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross-reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA</td>
<td>100 %</td>
</tr>
<tr>
<td>BPA-Glucuronide</td>
<td>85.0 %</td>
</tr>
<tr>
<td>BPA-Na-Sulfate</td>
<td>68.0 %</td>
</tr>
<tr>
<td>Bisphenol B</td>
<td>8.3 %</td>
</tr>
<tr>
<td>Bisphenol F</td>
<td>0.2 %</td>
</tr>
<tr>
<td>Diethylstilbestrol</td>
<td></td>
</tr>
<tr>
<td>Hexestrol</td>
<td></td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td></td>
</tr>
<tr>
<td>4-Heptylphenol</td>
<td></td>
</tr>
<tr>
<td>4-n-Nonylphenol</td>
<td>≦ 0.02 %</td>
</tr>
<tr>
<td>4-Propylphenol</td>
<td></td>
</tr>
<tr>
<td>4-Hexylsulphophenol</td>
<td></td>
</tr>
<tr>
<td>4-Pentyphenol</td>
<td></td>
</tr>
<tr>
<td>4-Hexyphenol</td>
<td></td>
</tr>
<tr>
<td>4-Butylphenol</td>
<td></td>
</tr>
<tr>
<td>2-ter-Butylphenol</td>
<td></td>
</tr>
<tr>
<td>4-Dodecylphenol</td>
<td></td>
</tr>
<tr>
<td>Di-n-Butyl-Phthalate</td>
<td></td>
</tr>
<tr>
<td>Benzyl-n-Butyl Phthalate</td>
<td></td>
</tr>
<tr>
<td>Daidzein</td>
<td></td>
</tr>
<tr>
<td>Genisteen</td>
<td></td>
</tr>
<tr>
<td>Bis-GMA</td>
<td></td>
</tr>
</tbody>
</table>

**STORAGE AND THE TERM OF VALIDITY**

Storage Condition: 2 - 8°C

The expiry date is specified on outer box.

**REFERENCE**


**PRECAUTION FOR INTENDED USE AND/OR HANDLING**

1. Bring each reagent and samples to room temperature and mix it to homogeneity without foaming.
2. “7, Stop solution” is a strong acid substance. Therefore, be careful not to have your skin and clothes contact “7, Stop solution” and pay attention to the disposal of “7, Stop solution”.
3. Since the measurement results are affected by the time and temperature of incubation, the samples and standards must be incubated under the same conditions.
4. “1, Precoated plate” and “3, Standard” contain sodium azide. Therefore, dispose these materials after diluting them with large quantity of water to avoid production of explosive metallic azide.
5. After testing, disposal must be made in accordance with national and local regulation separating the infectious waste.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from a different lot or kit.
8. All reagents should be stored at 2 - 8°C and do not use expired reagents. Put unused precoated plate strips into a sealed bag with a desiccant, and use by expiration date.
9. This kit is for research purpose only. Do not use for clinical diagnosis.
10. Handle samples as if there is capable of infection (HBVirus, HIV). The implements used for the assay should be treated in 0.1% sodium hypochlorite solution or autoclave.

**NAVIGATION**

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