Endothelin-1 Assay Kit - IBL

Please read carefully this instruction prior you use this assay kit.

INSTRUCTIONS FOR USE

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

KIT COMPONENT

1 Precoated plate: (Anti-Endothelin-1 Rabbit IgG A.P.) 96Well x 1
2 Labeled antibody: (Endothelin-1 peptide) 0.5mL x 1
3 Standard: (Endothelin-1 peptide) 10.5mL x 1
4 EIA buffer 30mL x 1
5 Solution for labeled antibody 12mL x 1
6 Chromogen: TMB solution 15mL x 1
7 Stop solution 12mL x 1
8 Wash buffer conc. 50mL x 1

MESURING SAMPLES

Serum, EDTA-plasma, Tissue extracted, Cell culture supernatant

PRINCIPLE

This kit is a solid phase sandwich ELISA (Enzyme-linked Immunosorbent Assay). As a primary antibody is coated on a plate, samples and standard are added into the wells for 1st reaction. After the reaction, HRP-conjugated secondary antibody is added into the wells for 2nd reaction. After washing away unbound the secondary antibody, Tetra Methyl Benzidine (TMB) is added to the wells and color develops.

OPERATING PRECATION

1 Test samples should be measured soon after collection. For storage of samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
2 Test samples should be diluted with "4, EIA buffer" contained in this kit.
3 Duplicate measurement of test samples and standards is recommended.
4 Standard curve should run for each assay.
5 Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
6 All reagents should be brought to room temperature (R.T.) and mixed completely and gently before use. After mixing them, make sure of no change in quality of the reagents.
7 Use only "8, Wash buffer conc.", contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
8 Fill the wash buffer each well, invert the plate and make sure the liquid is completely removed by shaking it off if you use a washing bottle. Repeat this washing process several times as instructed in order to avoid any insufficient washing process.
9 After remove the wash buffer, tapping the plate against a clean paper towel for completely removing the liquid from the wells and make sure the paper towel is not contact with inside of the wells in this process.
10 "6, Chromogen - TMB solution" should be stored in the dark due to its sensitivity against light. It should be also avoided contact with metals. Required quantity should be prepared into a collecting container for each use.
11 After adding TMB solution into the wells, the liquid in the wells gradually changes the color in blue. In this process the plate should be in dark. Remained TMB solution in the collecting container should not be returned into the original bottle of TMB solution to avoid contamination.
12 Measurement of O.D. should be done within 30 minutes after addition of "7, Stop solution".

OPERATION MANUAL AND DOSAGES

1. Materials needed but not supplied.
   Plate reader
   Test tubes for dilution
   Deionized water
   Paper towel
   Incubator (37°C±1°C)
   Micropipette and tip
   Measuring cylinder and beaker
   Plate washer
   Collecting container
   (i.e. clean disposable test tube)
   Refrigerator

2. Preparation
   (1) Preparation of wash buffer
      Dilute "8, Wash buffer conc." 40 fold with deionized water. The diluted one is used for the assay as a wash buffer. Adjust the required quantities if needed.
   (2) Preparation of labeled antibody

MEASUREMENT PROCEDURE

(1) Add test sample blank
   Determine tests for test sample blank. Put 100μL each of "4, EIA buffer" into the wells.
(2) Add prepared test samples and standard
   Put 100 μL prepared test samples and 100 μL prepared standard into appropriate wells.
(3) Incubation with plate lid (1st reaction).
(4) Washing
   Wash the plate with the prepared wash buffer and remove all liquid.
(5) Add prepared labeled antibody
   Put 100 μL prepared labeled antibody into the wells.
(6) Incubation with plate lid (2nd reaction).
(7) Washing
   Wash the plate with the prepared wash buffer and remove all liquid completely.
(8) Add "6, Chromogen - TMB solution"
   Put 100 μL the TMB solution into the wells.
(9) Incubation in dark
   (10) Add "7, Stop solution"
   Put 100 μL the Stop solution into the wells.

   (11) Determination of optical density (O.D.)
   Remove any dirt or drop of water on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, measure the both O.D. of standard and the test samples against a test sample blank.
   Measurement wavelength: 450 nm. In case of 2 wavelengths: Main wavelength is 450nm. Sub-wavelength is between 600 and 650 nm.

   Table for measurement procedure

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Test samples</th>
<th>Standard</th>
<th>Test sample blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test samples</td>
<td>100 μL</td>
<td>Diluted Standard</td>
<td>EIA buffer 100 μL</td>
</tr>
<tr>
<td>Incubation for overnight 2-8°C with plate lid.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Washing</td>
<td>5 times (wash buffer more than 350 μL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Labeled antibody</td>
<td>100 μL</td>
<td>100 μL</td>
<td>100 μL</td>
</tr>
<tr>
<td>Incubation for 30 minutes at 37°C with plate lid.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Washing</td>
<td>5 times (wash buffer more than 350 μL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMB solution</td>
<td>100 μL</td>
<td>100 μL</td>
<td>100 μL</td>
</tr>
<tr>
<td>Incubation for 30 minutes at R.T. (shielded).</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stop solution</td>
<td>100 μL</td>
<td>100 μL</td>
<td>100 μL</td>
</tr>
<tr>
<td>Measuring O.D.</td>
<td>450 nm / 600~655 nm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

   CALCULATION OF TEST RESULT
   1. Plot the concentration of the standard on the x-axis and its O.D. on the y-axis. Draw a standard curve by applying appropriate regression curve on each plot (i.e. quadratic regression of double logarithm conversion).
   2. Read the concentration by applying the absorbance of the test samples on a standard curve.

Manufacturer: Immuno-Biological Laboratories Co., Ltd.

URL: http://www.ibl-japan.co.jp  E-mail: do-ibl@ibl-japan.co.jp
**PERFORMANCE AND CHARACTERISTICS**

1 Sensitivity

0.39 pg/mL

2 Measurement range

0.78 – 100 pg/mL

3 Dilution linearity

<table>
<thead>
<tr>
<th>Test samples</th>
<th>Theoretical value (pg/mL)</th>
<th>Measurement value (pg/mL)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Added 10%FCS</td>
<td>25</td>
<td>26.51</td>
<td>106.0</td>
</tr>
<tr>
<td>RPMI-1640 Media</td>
<td>4</td>
<td>50</td>
<td>100.2</td>
</tr>
<tr>
<td>Serum (Healthy subject)</td>
<td>2</td>
<td>4</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>25</td>
<td>94.2</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>12.5</td>
<td>91.7</td>
</tr>
</tbody>
</table>

4 Added recovery assay

<table>
<thead>
<tr>
<th>Test samples</th>
<th>Theoretical value (pg/mL)</th>
<th>Measurement value (pg/mL)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Added 10%FCS</td>
<td>50</td>
<td>50.11</td>
<td>100.2</td>
</tr>
<tr>
<td>Serum (Healthy subject)</td>
<td>12.5</td>
<td>13.16</td>
<td>105.3</td>
</tr>
<tr>
<td></td>
<td>6.25</td>
<td>5.89</td>
<td>94.2</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>20.36</td>
<td>81.4</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>10.66</td>
<td>85.3</td>
</tr>
<tr>
<td></td>
<td>6.25</td>
<td>5.13</td>
<td>82.1</td>
</tr>
<tr>
<td></td>
<td>3.13</td>
<td>2.87</td>
<td>91.7</td>
</tr>
</tbody>
</table>

5 Intra-assay

<table>
<thead>
<tr>
<th>Measurement value (pg/mL)</th>
<th>SD (pg/mL)</th>
<th>CV (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>55.39</td>
<td>1.82</td>
<td>3.3</td>
<td>8</td>
</tr>
<tr>
<td>9.95</td>
<td>0.29</td>
<td>2.9</td>
<td>8</td>
</tr>
<tr>
<td>5.64</td>
<td>0.15</td>
<td>2.7</td>
<td>8</td>
</tr>
</tbody>
</table>

6 Inter-assay

<table>
<thead>
<tr>
<th>Measurement value (pg/mL)</th>
<th>SD (pg/mL)</th>
<th>CV (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>56.19</td>
<td>1.19</td>
<td>2.1</td>
<td>24</td>
</tr>
<tr>
<td>9.68</td>
<td>0.29</td>
<td>3.0</td>
<td>24</td>
</tr>
<tr>
<td>5.67</td>
<td>0.14</td>
<td>2.5</td>
<td>24</td>
</tr>
</tbody>
</table>

7 Specificity

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelin-1(1-21)</td>
<td>100</td>
</tr>
<tr>
<td>Endothelin-2(1-21)</td>
<td>3.32</td>
</tr>
<tr>
<td>Endothelin-3(1-21)</td>
<td>≤0.1</td>
</tr>
<tr>
<td>Endothelin-1(1-31)</td>
<td>0.16</td>
</tr>
<tr>
<td>Endothelin-2(1-31)</td>
<td>≤0.1</td>
</tr>
<tr>
<td>Endothelin-3(1-31)</td>
<td>≤0.1</td>
</tr>
<tr>
<td>Big Endothelin-1</td>
<td>≤0.1</td>
</tr>
<tr>
<td>Big Endothelin-2</td>
<td>≤0.1</td>
</tr>
<tr>
<td>Big Endothelin-3</td>
<td>≤0.1</td>
</tr>
<tr>
<td>VIC (Mouse Endothelin-2)</td>
<td>≤0.1</td>
</tr>
<tr>
<td>Rat Big Endothelin-1</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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

1 Precaution for handling (Hazard prevention)

(1) Treat the components carefully and wash hands after handling it.
(2) “7, Stop solution” is a strong acid substance (1N Sulfuric acid). Therefore, it should be careful for the treatment and do not contact your skin and clothes with it. It also needs to pay attention to the disposal of it.

2 Precaution for intended use

(1) “2. Labeled antibody.” And “3. Standard” are lyophilized products. It should be careful to open this vial.
(2) Should be stored at -20°C.
(3) Precipitation can be seen in “4, EIA buffer” and “8, Wash buffer conc.”, however, it does not affect its performance.
(4) Do not mix or replace the reagents with the reagents from a different lot or kit.
(5) Do not use expired reagents.

3 Precaution for disposal

(1) Dispose used materials after rinsing them with large quantity of water.

**Attention for sample handling:**

This kit will allow a direct assay samples containing a low concentration of protein (e.g. cell culture media, urine and so on). However, extraction and concentration of Endothelin from samples will be required for samples containing a high concentration of protein (e.g. plasma, serum, tissue homogenates and so on). Extraction of test sample with Sep-Pak C-18 column is recommended as below:

1. Pre-treatment of Sep-Pak C-18 column (*1)
   a. Washing with 4mL of pure methanol.
   b. Washing 2 times with 2mL of distilled water.
   c. Washing 2 times with 2mL of 0.1% TFA solution.

2. Pre-treatment of samples
   a. Plasma (serum) – Addition of 6mL of 10% CH₃COOH to 2mL of plasma with mixing
   b. Tissue sample
     (1) Addition of 1M CH₃COOH - 20mM HCl solution to tissue sample and homogenize.
     (2) After boiling for ten minutes, centrifuge at 10,000rpm for 10min and collecting a supernatant.

3. Extraction of sample
   a. Addition of treated sample to Sep-Pak C-18 column.
   b. Washing 3 times with 3mL of distilled water.
   c. Elution with 2mL of an appropriate solution (*2) and collection to vial

4. Measurement

Collected sample in vial should be lyophilized and stored under frozen condition until measurement. Stored sample should be reconstituted with 0.1mL of an appropriate solution (*3) and added 0.2mL of “4, EIA buffer” and mixed. Confirm that the pH of sample is in a neutral range before measurement. There is a difference in recovery rate between samples. Please test added recovery assay in advance.

(*) Product No. WAT023501, manufactured by Waters. (U.S.A.)
(2) 0.1% Trifluoroacetic Acid (4) plus 60% Acetonitrile in dH₂O
(3) 0.1% Trifluoroacetic Acid in DMSO
(4) No. 206-10731, manufactured by Wako Pure Chemical Industries Ltd. (Japan) is used in our protocol.

**STORAGE AND THE TERM OF VALIDITY**

Storage Condition: -20°C

The expiry date is specified on the outer box.

**PACKAGE UNIT AND PRODUCT NUMBER**

Package unit: 96 Well
Product number: 17165

**REFERENCES**


**CONTACT DETAILS**

Distributed By: IBL-America, Inc.
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(888) 523 1246

Manufacturer: Immuno-Biological Laboratories Co., Ltd.