Human Titin N-Fragment 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-Titin-N(144A2) Mouse IgG) 96 Well x 1
2. Labeled antibody conc.: (50X) HRP conjugated Anti-Titin-N(53A1) Mouse IgG) 0.4 mL x 1
3. Standard: (Recombinant Human Titin (1-200)) 0.5 mL x 2
4. EIA buffer: 30 mL x 1
5. Solution for labeled antibody: 12 mL x 1
6. Chromogen: TMB solution: 15 mL x 1
7. Stop solution: 12 mL x 1
8. Wash buffer conc.: 50 mL x 1

MEASURING SAMPLES

Human Urine

OPERATING PRECAUTION

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. Using a plate washer is recommended (wait time zero second). It should be washed by a plate washer immediately after each reaction. If you use a washing bottle instead of a plate washer, after filling wash buffer in each well, immediately turn the plate upside down and shake it off to completely remove the wash buffer. Repeat the number of times of wash defined in a table for measurement procedure described in section 3. It should be properly washed off as instructed in order to avoid any insufficient wash.
9. Carefully tap the plate against a clean paper towel without contacting with inside of each well to completely remove the washing buffer after repeated the determined number of wash.
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".

OPERATING MANUAL AND DOSAGES

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

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
     Dilute "2. Labeled antibody conc." 30 fold with "5. Solution for labeled antibody" using a prepared collecting container.

3. Measurement Procedure
   - (1) Add test sample blank
     Dilute test samples with "4. EIA buffer" contained in this kit as follows. Human Urine: more than 10 fold.
   - (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 (Refer to No. 8 and 9 described in OPERATING PRECAUTION.)
     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 (Refer to No. 8 and 9 described in OPERATING PRECAUTION.)
     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>Test samples</th>
<th>Standard</th>
<th>Test sample blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagents</td>
<td>Test samples 100 μL</td>
<td>Diluted Standard 100 μL</td>
</tr>
<tr>
<td>1st reaction</td>
<td>Incubation for 60 minutes at 37°C with plate lid.</td>
<td></td>
</tr>
<tr>
<td>Washing</td>
<td>4 times (wash buffer more than 350 μL) (Refer to No. 8 and 9 described in OPERATING PRECAUTION.)</td>
<td></td>
</tr>
<tr>
<td>Labeled antibody</td>
<td>100 μL</td>
<td>100 μL</td>
</tr>
<tr>
<td>2nd reaction</td>
<td>Incubation for 30 minutes at 37°C with plate lid.</td>
<td></td>
</tr>
<tr>
<td>Washing</td>
<td>5 times (wash buffer more than 350 μL) (Refer to No. 8 and 9 described in OPERATING PRECAUTION.)</td>
<td></td>
</tr>
<tr>
<td>TMB solution</td>
<td>100 μL</td>
<td>100 μL</td>
</tr>
<tr>
<td>Chromogenic reaction</td>
<td>Incubation for 30 minutes at R.T. (shielded)</td>
<td></td>
</tr>
<tr>
<td>Stop solution</td>
<td>100 μL</td>
<td>100 μL</td>
</tr>
<tr>
<td>Measuring O.D.</td>
<td>450 nm / 660~650 nm</td>
<td></td>
</tr>
</tbody>
</table>

Manufacturer: Immuno-Biological Laboratories Co., Ltd.
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.

3. Calculate the concentration of the test samples by multiplying dilution ratio of test samples on the value.

Example of standard curve and measured value

<table>
<thead>
<tr>
<th>Standard (pmol/L)</th>
<th>O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3000.0</td>
<td>2.975</td>
</tr>
<tr>
<td>1500.0</td>
<td>1.510</td>
</tr>
<tr>
<td>750.0</td>
<td>0.719</td>
</tr>
<tr>
<td>375.0</td>
<td>0.340</td>
</tr>
<tr>
<td>187.5</td>
<td>0.165</td>
</tr>
<tr>
<td>93.75</td>
<td>0.081</td>
</tr>
<tr>
<td>46.88</td>
<td>0.040</td>
</tr>
</tbody>
</table>

PERFORMANCE AND CHARACTERISTICS

1 Sensitivity
27.91 pmol/L (Calculated by NCCLS method using the standard.)

2 Measurement range
46.88 ~ 3000 pmol/L

3 Dilution linearity

Dilution linearity of Human Urine

<table>
<thead>
<tr>
<th>OD 450</th>
<th>Titin N-Fragment (pmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3000</td>
<td>2.975</td>
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4 Added recovery assay

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Additive Amount (pmol/L)</th>
<th>Theoretical Value (pmol/L)</th>
<th>Measurement Value (pmol/L)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Urine (x10)</td>
<td>1389.2</td>
<td>1572.9</td>
<td>1623.4</td>
<td>103.2</td>
</tr>
<tr>
<td></td>
<td>432.0</td>
<td>615.7</td>
<td>540.5</td>
<td>87.8</td>
</tr>
<tr>
<td></td>
<td>131.0</td>
<td>314.7</td>
<td>313.4</td>
<td>99.6</td>
</tr>
</tbody>
</table>

5 Intra-assay

<table>
<thead>
<tr>
<th>Measurement value (pmol/L)</th>
<th>SD (pmol/L)</th>
<th>CV (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1189.07</td>
<td>44.97</td>
<td>3.8</td>
<td>24</td>
</tr>
<tr>
<td>358.61</td>
<td>15.23</td>
<td>4.2</td>
<td>24</td>
</tr>
<tr>
<td>102.76</td>
<td>8.85</td>
<td>8.6</td>
<td>24</td>
</tr>
</tbody>
</table>

6 Inter-assay

<table>
<thead>
<tr>
<th>Measurement value (pmol/L)</th>
<th>SD (pmol/L)</th>
<th>CV (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1188.21</td>
<td>68.02</td>
<td>5.7</td>
<td>7</td>
</tr>
<tr>
<td>364.99</td>
<td>20.79</td>
<td>5.7</td>
<td>7</td>
</tr>
<tr>
<td>107.61</td>
<td>8.27</td>
<td>7.7</td>
<td>7</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) “3, Standard” is lyophilized products. It should be careful to open this vial.
(2) All reagents should be stored at 2 - 8°C.
(3) Precipitation can be seen in “4, EIA buffer”, “5, Solution for labeled antibody” 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.

STORAGE AND THE TERM OF VALIDITY

Storage Condition: 2 - 8°C
The expiry date is specified on the outer box.

PACKAGE UNIT AND PRODUCT NUMBER

Package unit: 96 Well
Product number: 27900

REFERENCE*


CONTACT DETAILS

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