Mouse 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(151A1) Mouse IgG.) 96Well x 1
2. Labeled antibody conc.: (30x) HRP conjugated Anti-Titin-N(144A2) Mouse IgG 0.4mL x 1
3. Standard: (Recombinant Mouse Titin (1-200)) 0.5mL x 2
4. EIA buffer 30mL x 1
5. Solution for labeled antibody 10mL x 1
6. Chromogen: TMB solution 15mL x 1
7. Stop solution 12mL x 1
8. Wash buffer conc. 50mL x 1

MEASURING SAMPLES

Mouse, Rat, Equine and Dog urine

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 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 washing buffer in each well, immediately turn the plate upside down and shake it off to completely remove the washing 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.

OPERATION 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.

Example)
In case you use one strip (8 well), the required quantity of Labeled antibody is 800 μL. (Dilute 30 μL of "2, Labeled antibody Conc." with 870 μL of "5, Solution for labeled antibody" and mix it. And use 100 μL (the mixed solution in each well.)

This operation should be done just before applying labeled antibody.

The remaining "2, Labeled antibody Conc." should be stored at 4°C in a firmly sealed vial.

(3) Preparation of standard
Add 0.5 mL of deionized water into the vial of "3, Standard" and completely dissolve it. Concentration of the standard is 9,600 pmol/L. The standards enclosed in this kit can be frozen and stored after reconstitution. However the freeze-thaw shall not be repeated.

Prepare 7 test tubes for dilution of the standard and adding 230 μL of the EIA buffer into each tube.

Put 230 μL of 9,600 pmol/L standard into the tube 4,800 pmol/L (Tube-1) and gently mix it. Afterward, put 230 μL of the mixed liquid of tube-1 into the tube 2,400 pmol/L (Tube-2) and gently mix it. Dilute two fold standard solution in series to set up 7 points of diluted standard between 4,800 pmol/L and 75 pmol/L.

<table>
<thead>
<tr>
<th>Table for measurement procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test samples</td>
</tr>
<tr>
<td>Reagents</td>
</tr>
<tr>
<td>100 μL</td>
</tr>
<tr>
<td>1st reaction</td>
</tr>
<tr>
<td>Washing</td>
</tr>
<tr>
<td>Labeled antibody</td>
</tr>
<tr>
<td>2nd reaction</td>
</tr>
<tr>
<td>Washing</td>
</tr>
<tr>
<td>TMB solution</td>
</tr>
<tr>
<td>Chromogenic reaction</td>
</tr>
<tr>
<td>Stop solution</td>
</tr>
<tr>
<td>Measuring O.D.</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

PERFORMANCE AND CHARACTERISTICS

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

2. Measurement range
   75 ~ 4,800 pmol/L

3. Dilution linearity

<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>Mouse Urine (x10)</td>
<td>2400</td>
<td>2603.7</td>
<td>2619.9</td>
<td>100.6</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>803.7</td>
<td>786.1</td>
<td>97.8</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>353.7</td>
<td>315.4</td>
<td>89.2</td>
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<td>Rat Urine (x10)</td>
<td>2400</td>
<td>2794.2</td>
<td>2827.8</td>
<td>101.2</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>994.2</td>
<td>963.3</td>
<td>96.9</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>544.2</td>
<td>540.6</td>
<td>99.3</td>
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<td>Equine Urine (x10)</td>
<td>2400</td>
<td>3333.3</td>
<td>2895.1</td>
<td>86.9</td>
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<td></td>
<td>600</td>
<td>1533.3</td>
<td>1239.5</td>
<td>80.8</td>
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<tr>
<td></td>
<td>150</td>
<td>1083.3</td>
<td>926.3</td>
<td>85.5</td>
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<td>Dog Urine (x10)</td>
<td>2400</td>
<td>3750.4</td>
<td>3041.8</td>
<td>81.1</td>
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<tr>
<td></td>
<td>600</td>
<td>1950.4</td>
<td>1593.4</td>
<td>81.7</td>
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<tr>
<td></td>
<td>150</td>
<td>1500.4</td>
<td>1428.8</td>
<td>95.2</td>
</tr>
</tbody>
</table>

4. Added recovery assay

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>207.2</td>
<td>54.1</td>
<td>2.6</td>
<td>24</td>
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<td>629.9</td>
<td>24.9</td>
<td>4.0</td>
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<tr>
<td>260.2</td>
<td>15.5</td>
<td>6.0</td>
<td>24</td>
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</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>2110.2</td>
<td>88.6</td>
<td>4.2</td>
<td>7</td>
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<tr>
<td>623.0</td>
<td>38.7</td>
<td>6.2</td>
<td>7</td>
</tr>
<tr>
<td>268.8</td>
<td>14.1</td>
<td>5.2</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, Step 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: 27602

CONTACT DETAILS

Manufacturer: Immuno-Biological Laboratories Co., Ltd.

Instruction for Use Code No. 27602
1st Version made in June 2021