OPERATING PRECAUTION

Antibody, Tetra Methyl Benzidine (TMB) is added to the wells and color develops.

KIT COMPONENT

1. Precoated plate: (Anti- Mouse LPL (23A1) Rat IgG A.P.) (Recombinant Mouse LPL) 96Well x 1  
2. Labeled antibody conc. (30X) HRP conjugated Anti-Mouse LPL (31A5) Rat IgG A.P. 0.4mL x 1  
3. Standard: (Solution for labeled antibody) 0.5mL x 2  
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

MEASURING SAMPLES

Mouse serum and mouse post heparin EDTA-plasma

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 the 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. Wash the plate immediately after each reaction using by a plate washer with stop solution. If you use a multichannel pipette or a washing bottle due to no availability of any plate washer, filling wash buffer in each well and setting wait time zero second. The O.D. value tends to be lower if washing time is getting longer. If you use a multi-channel pipette or a washing bottle due to no availability of any plate washer, filling wash buffer in each well and 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”.

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
   - Paper towel
   - Collecting container (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) Preparation of standard
   - Add 0.5 mL of “4, EIA buffer” into the vial of “3, Standard” and completely dissolve it. Concentration of the standard is 40 ng/mL. 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 40 ng/mL standard into the tube 20 ng/mL (Tube-1) and gently mix it. Afterword, put 230 μL of the mixed liquid of tube-1 into the tube 10 ng/mL (Tube-2) and gently mix it. Dilute two fold standard solution in series to set up 7 points of diluted standard between 20 ng/mL and 0.31 ng/mL.
   - The standards enclosed in this kit can be frozen and stored after reconstitution. However the freeze-thaw shall not be repeated.

   (4) Preparation of test samples
   - Dilute test samples with “4, EIA buffer” contained in this kit as follows. Mouse serum: 50-fold
   - Mouse post heparin EDTA-plasma: 250-fold

3 MEASUREMENT PROCEDURE

1. Add test sample blank
   Determine wells 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>
<thead>
<tr>
<th>Reagents</th>
<th>Test samples</th>
<th>Standard</th>
<th>EIA buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test samples 100 μL</td>
<td>Diluted Standard 100 μL</td>
<td>EIA buffer 100 μL</td>
<td></td>
</tr>
</tbody>
</table>

1. Preparation
   Incubation for overnight at 2~8°C with plate lid.

2. Washing
   4 times (wash buffer more than 350 μL) (Refer to No. 6 and 9 described in OPERATING PRECAUTION.)

3. Labeled antibody
   100 μL

4. Washing
   5 times (wash buffer more than 350 μL) (Refer to No. 6 and 9 described in OPERATING PRECAUTION.)

5. TMB solution
   100 μL

6. Chromogenic reaction
   Incubation for 30 minutes at R.T. (shielded).

7. Stop solution
   100 μL

8. Measuring O.D.
   450 nm / 600—650 nm

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 (ng/mL)</th>
<th>O.D. (450nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.00</td>
<td>3.210</td>
</tr>
<tr>
<td>10.00</td>
<td>1.625</td>
</tr>
<tr>
<td>5.00</td>
<td>0.778</td>
</tr>
<tr>
<td>2.50</td>
<td>0.384</td>
</tr>
<tr>
<td>1.25</td>
<td>0.192</td>
</tr>
<tr>
<td>0.63</td>
<td>0.096</td>
</tr>
<tr>
<td>0.31</td>
<td>0.052</td>
</tr>
</tbody>
</table>

PERFORMANCE AND CHARACTERISTICS

1. Sensitivity
   0.036 ng/mL (Calculated by NCCLS method using the standard.)

2. Measurement range
   0.31 ~ 20 ng/mL

3. Dilution linearity

   4 Added recovery assay

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Additive Amount (ng/mL)</th>
<th>Theoretical Value (ng/mL)</th>
<th>Measurement Value (ng/mL)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse Serum (x50)</td>
<td>2.50</td>
<td>5.69</td>
<td>4.77</td>
<td>83.8</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>4.44</td>
<td>4.03</td>
<td>90.8</td>
</tr>
<tr>
<td></td>
<td>0.63</td>
<td>3.82</td>
<td>3.60</td>
<td>94.2</td>
</tr>
<tr>
<td>Mouse Post heparin EDTA Plasma (x250)</td>
<td>2.50</td>
<td>12.48</td>
<td>13.09</td>
<td>104.9</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>11.23</td>
<td>11.69</td>
<td>104.1</td>
</tr>
<tr>
<td></td>
<td>0.63</td>
<td>10.60</td>
<td>10.80</td>
<td>101.9</td>
</tr>
</tbody>
</table>

5. Intra-assay

<table>
<thead>
<tr>
<th>Measurement value (ng/mL)</th>
<th>SD (ng/mL)</th>
<th>CV (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.28</td>
<td>0.37</td>
<td>4.0</td>
<td>24</td>
</tr>
<tr>
<td>2.00</td>
<td>0.12</td>
<td>6.0</td>
<td>24</td>
</tr>
<tr>
<td>0.67</td>
<td>0.07</td>
<td>10.4</td>
<td>24</td>
</tr>
</tbody>
</table>

6. Inter-assay

<table>
<thead>
<tr>
<th>Measurement value (ng/mL)</th>
<th>SD (ng/mL)</th>
<th>CV (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.31</td>
<td>0.63</td>
<td>6.1</td>
<td>12</td>
</tr>
<tr>
<td>2.24</td>
<td>0.12</td>
<td>5.4</td>
<td>12</td>
</tr>
<tr>
<td>0.73</td>
<td>0.04</td>
<td>5.5</td>
<td>12</td>
</tr>
</tbody>
</table>

7. Specificity

   Specifically detect Mouse Lipoprotein Lipase (LPL) in mouse serum and mouse post heparin EDTA-plasma.

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: 27603

CONTACT DETAILS

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