Apolipoprotein B100 ELISA

Enzyme immunoassay for the quantitative determination of human Apolipoprotein B100 (ApoB-100) in serum, EDTA-plasma and cell culture supernatant

REF JP27181

12 x 8

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Distributed by:

IBL INTERNATIONAL GMBH
Flughafenstrasse 52a
D-22335 Hamburg, Germany
Phone: +49 (0)40-53 28 91-0
Fax: +49 (0)40-53 28 91-11
IBL@IBL-International.com
www.IBL-International.com
Human ApoB-100 Assay Kit - IBL

INTRODUCTION

Though the lipids (such as fat and cholesterol) are not soluble in water, apolipoproteins are carrier proteins that combine with lipids to form lipoprotein particles which are water-soluble and can be carried through water-based circulation (i.e., blood, lymph). Apolipoproteins are classified according to their forms and functions, and there are six major classes and several sub-classes. ApoB (Apolipoprotein B) is the primary apolipoprotein of LDL and is considered to reflect change of LDL level in blood. There are two types apolipoprotein in ApoB. One is ApoB-100 which correlates with cholesterol level well and is released as LDL, and another is ApoB-48, which presents being incorporated into chylomicrons and correlates well with triglyceride.

Generally, the concentration of ApoB in blood has been measured as the total of ApoB-100 and ApoB-48. This kit can measure only ApoB-100 in plasma samples using ApoB-100 specific antibodies which don’t recognize ApoB-48. This highly sensitive sandwich ELISA can also measure ApoB-100 in cell culture media or various fractionated lipoprotein samples.

PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of highly specific antibodies. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The strength of coloring is proportional to the quantities of Human ApoB-100.

MEASUREMENT RANGE

0.13 - 8.4 μg/mL

INTENDED USE

For research use only. Not for use in diagnostic procedures.

This IBL’s assay kit is capable for the quantitative determination of Human ApoB-100 in serum, EDTA-plasma and cell culture media.

The guide line of dilution rate for serum and plasma samples is more than 500-fold with "4, EIA buffer". However, optimal dilution should be examined by each experiment.

KIT COMPONENT

1. Precocated plate : Anti-Human ApoB-100 Rabbit IgG Affinity Purify 96Well x 1
2. Labeled antibody Conc. : (30X)HRP conjugated Anti-Human ApoB-100 (35B1) Mouse IgG Fab’ Affinity Purify 0.4mL x 1
3. Standard : Human ApoB-100 0.5mL x 2
4. EIA buffer : 1% BSA, 0.05% Tween20 in PBS 50mL x 1
5. Solution for Labeled antibody : 1% BSA, 0.05% Tween20 in PBS 12mL x 1
6. Chromogen : TMB solution 15mL x 1
7. Stop solution : 1N H₂SO₄ 50mL x 1
8. Wash buffer Conc. : (40X) 0.05% Tween20 in phosphate buffer 50mL x 1

OPERATION MANUAL

1. Materials needed but not supplied
   - Plate reader (450nm)
   - Incubator (37°C ± 1°C)
   - Micro pipette and tip
   - Graduated cylinder and beaker
   - Refrigerator (as 4°C)
   - Paper towel
   - Plate for dilution of serum or plasma
   - Washing bottle for precoated plate
   - Deionized water

2. Preparation

1) Preparation of wash buffer
   "8. Wash buffer Conc." is a concentrated (40X) 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. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.

2) Preparation of Labeled antibody
   "2. Labeled antibody Conc." is a concentrated (30X). Dilute "2, Labeled antibody Conc." with "5, Solution for Labeled antibody" in 30 times according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody. Example)
   In case you use one strip (8 well), the required quantity of Labeled antibody is 900 μL. Dilute 30 μL of "2. Labeled antibody Conc." with 870 μL of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100 μL in each well.

This operation should be done just before the applying Labeled antibody. The remaining "2, Labeled antibody Conc." should be stored at 4°C in firmly sealed vial.

3) Preparation of Standard
   Put just 0.5 mL of deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 18.8 μg/mL Human ApoB-100 standard.

4) Dilution of Standard
   Prepare 8 tubes for dilution of "3, Standard". Put 230 μL each of "4, EIA buffer" into the tube. Specify the following concentration of each tube.
   - Tube-1 8.4 μg/mL
   - Tube-2 4.2 μg/mL
   - Tube-3 2.1 μg/mL
   - Tube-4 1.05 μg/mL
   - Tube-5 0.53 μg/mL
   - Tube-6 0.26 μg/mL
   - Tube-7 0.13 μg/mL
   - Tube-8 0 μg/mL (Test Sample Blank)

Put 230 μL of Standard solution into tube-1 and mix it gently. Then, put 230 μL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 8.4 μg/mL and 0.13 μg/mL. Tube-8 is the test sample blank as 0 μg/mL.

See following picture.

5) Dilution of test sample
   Serum or plasma samples have to be diluted with "4, EIA buffer" accordingly. The recommended dilution for them is more than 500-fold. In case of the absorbance of sample is over than the assay range, it is necessary to dilute it more.
   "Example of 500-fold dilution of serum or plasma"
   1. Add 20 μL of serum or plasma to 380 μL of "4, EIA buffer" in a tube and mix them well.
   2. Pipette 20 μL of 20-fold diluted serum or plasma from the tube of above first dilution and add it to 480 μL of "4, EIA buffer" in another tube, and mix them well.
   3. This 500-fold diluted serum or plasma should be applied as a test sample according to the measurement procedure.

If the concentration of human ApoB-100 in samples may not be estimated in advance, the pre-assay with several different dilutions will be recommended to determine the proper dilution of samples.

3. Measurement procedure

All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Make sure of no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples.

Put the plate at 450nm against a Reagent Blank within 30 minutes after addition of stop solution.

Put 230 μL of Standard solution into tube-1 and mix it gently. Then, put 230 μL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 8.4 μg/mL and 0.13 μg/mL. Tube-8 is the test sample blank as 0 μg/mL.

See following picture.

Put 230 μL of Standard solution into tube-1 and mix it gently. Then, put 230 μL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 8.4 μg/mL and 0.13 μg/mL. Tube-8 is the test sample blank as 0 μg/mL.

Put 230 μL of Standard solution into tube-1 and mix it gently. Then, put 230 μL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 8.4 μg/mL and 0.13 μg/mL. Tube-8 is the test sample blank as 0 μg/mL.

See following picture.

Put 230 μL of Standard solution into tube-1 and mix it gently. Then, put 230 μL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 8.4 μg/mL and 0.13 μg/mL. Tube-8 is the test sample blank as 0 μg/mL.

See following picture.

Put 230 μL of Standard solution into tube-1 and mix it gently. Then, put 230 μL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 8.4 μg/mL and 0.13 μg/mL. Tube-8 is the test sample blank as 0 μg/mL.

See following picture.
**SPECIAL ATTENTION**

1. Test samples should be measured soon after collection. For the storage of test 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. Dilution linearity of EDTA-Plasma

3. Duplicate measurement of test samples and standard is recommended.

4. Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.

5. Wash hands after handling reagents.

6. Wash the wash buffer completely by tapping the precoated plate on paper towel. Do not wipe wells with paper towel.

7. "3, Standard" is lyophilized products. Be careful to open this vial.

8. Dispose used materials after rinsing them with large quantity of water.

9. This kit is for research purpose only. Do not use for clinical diagnosis.

**CALCULATION OF TEST RESULT**

Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting. Plot the subtracted absorbance of the standards against the standard concentration on log-log graph paper. Draw the best smooth curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

**Example of standard curve**

<table>
<thead>
<tr>
<th>Conc. (μg/mL)</th>
<th>Absorbance (450nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>0.009</td>
</tr>
<tr>
<td>0.1</td>
<td>0.01</td>
</tr>
<tr>
<td>1</td>
<td>0.02</td>
</tr>
<tr>
<td>2</td>
<td>0.03</td>
</tr>
<tr>
<td>5</td>
<td>0.05</td>
</tr>
<tr>
<td>10</td>
<td>0.1</td>
</tr>
</tbody>
</table>

* The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

**PERFORMANCE CHARACTERISTICS**

1. **Dilution linearity**

2. **Added Recovery Assay**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Theoretical Value (μg/mL)</th>
<th>Measured Value (μg/mL)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%FCS added RPMI-1640</td>
<td>4.20</td>
<td>4.57</td>
<td>108.8</td>
</tr>
<tr>
<td></td>
<td>2.10</td>
<td>2.33</td>
<td>111.0</td>
</tr>
<tr>
<td></td>
<td>1.05</td>
<td>1.12</td>
<td>106.7</td>
</tr>
<tr>
<td></td>
<td>0.53</td>
<td>0.54</td>
<td>101.9</td>
</tr>
<tr>
<td></td>
<td>0.26</td>
<td>0.25</td>
<td>98.2</td>
</tr>
<tr>
<td>Human Serum (x400)</td>
<td>7.78</td>
<td>7.57</td>
<td>97.3</td>
</tr>
<tr>
<td></td>
<td>5.68</td>
<td>5.88</td>
<td>103.5</td>
</tr>
<tr>
<td></td>
<td>4.63</td>
<td>4.74</td>
<td>102.4</td>
</tr>
<tr>
<td></td>
<td>4.11</td>
<td>4.26</td>
<td>103.6</td>
</tr>
<tr>
<td></td>
<td>3.84</td>
<td>3.88</td>
<td>101.0</td>
</tr>
<tr>
<td></td>
<td>3.88</td>
<td>3.71</td>
<td>97.1</td>
</tr>
<tr>
<td>Human Plasma (x400)</td>
<td>5.89</td>
<td>5.47</td>
<td>92.9</td>
</tr>
<tr>
<td></td>
<td>4.94</td>
<td>4.54</td>
<td>93.8</td>
</tr>
<tr>
<td></td>
<td>4.32</td>
<td>3.98</td>
<td>92.1</td>
</tr>
<tr>
<td></td>
<td>4.05</td>
<td>3.80</td>
<td>93.8</td>
</tr>
</tbody>
</table>

3. **Intra - Assay**

<table>
<thead>
<tr>
<th>Mean Value (μg/mL)</th>
<th>SD (μg/mL)</th>
<th>CV (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.69</td>
<td>1.17</td>
<td>3.6</td>
<td>26</td>
</tr>
<tr>
<td>2.62</td>
<td>0.12</td>
<td>4.6</td>
<td>26</td>
</tr>
<tr>
<td>0.90</td>
<td>0.05</td>
<td>5.6</td>
<td>26</td>
</tr>
</tbody>
</table>

4. **Cross-Recognitiy**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross-Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human ApoB-100</td>
<td>100 %</td>
</tr>
<tr>
<td>Human ApoB-48</td>
<td>&lt; 0.1 %</td>
</tr>
</tbody>
</table>

5. **Sensitivity**

   0.03 μg/mL

6. **Precaution for Intended Use and/or Handling**

   1. All reagents should be stored at 2 - 8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
   
   2. "3, Standard" is lyophilized products. Be careful to open this vial.
   
   3. "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".
   
   4. Precipitation may occur in "2, Labeled antibody Conc." or "4, EIA buffer" however, there is no problem in the performance.
   
   5. Wash hands after handling reagents.
   
   6. Wash hands after handling reagents.
   
   7. Do not mix the reagents with the reagents from a different lot or kit.
   
   8. Do not use expired reagents.

**STORAGE AND THE TERM OF VALIDITY**

Storage Condition : 2 - 8°C

The expiry date is specified on outer box.

**Version 1.**

Made in Japan.

**Instructions Code No. 27181**

**For research use only. Not for use in diagnostic procedures.**
<table>
<thead>
<tr>
<th>REF</th>
<th>Cat.-No.: / Cat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Use by: / Verwendbar bis: / Utiliser à: / Usado até: / Da utilizzare entro: / Χρησιμοποιείται από:</td>
</tr>
</tbody>
</table>