Code No. 27181

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 (ApolipoproteinB) 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 VLDL. Another is ApoB-48 which presents being incorporated into chylomicrons and correlates well with tryglyceride.
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	Precoated 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*		50mL 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

OPERATION MANUAL

1. Materials needed but not supplied

- Plate reader (450nm)
- Micropipette and tip
- Graduated cylinder and beaker
- Deionized water
- Refrigerator (as 4°C)
- Graph paper (log/log)
- Paper towel
- Tube for dilution of Standard
- Incubator (37°C ± 1°C)
- Tube for dilution of serum or plasma
- Washing bottle for precoated plate
- Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"

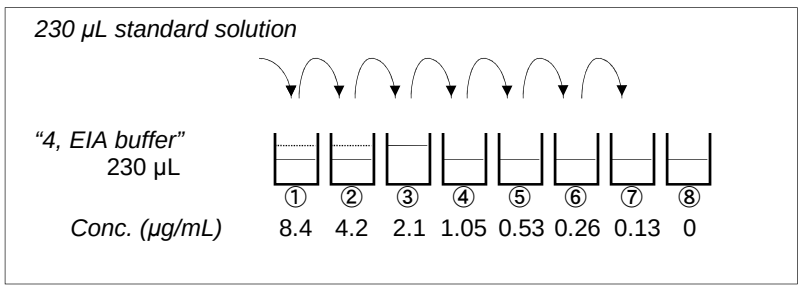
2. Preparation

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 800 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.)
<u>This operation should be done just before the applying Labeled antibody.</u>
The remaining "2, Labeled antibody Conc." should be stored at 4°C in firmly sealed vial.
3) Preparation of Standard
Put just <u>0.5 mL</u> of deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 16.8 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
<u>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.</u>

<u><Example of 500-fold dilution of serum or plasma></u>
1. Add 20 µL of serum or plasma to 380µL 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 480µL 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.

Reagents	Test Sample	Standard	Test Sample Blank	Reagent Blank
	Test sample 100 µL	Diluted standard (Tube 1-7) 100 µL	EIA buffer (Tube-8) 100 µL	EIA buffer 100 µL
Incubation for 60 minutes at 37°C with plate lid				
3 times (wash buffer more than 350 µL) *				
Labeled Antibody	100 µL	100 µL	100 µL	-
Incubation for 30 minutes at 4°C with plate lid				
4 times (wash buffer more than 350 µL) *				
Chromogen	100 µL	100 µL	100 µL	100 µL
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 µL	100 µL	100 µL	100 µL
Read the plate at 450nm against a Reagent Blank within 30 minutes after addition of Stop solution.				

1) Determine wells for reagent blank. Put 100 µL each of "4, EIA buffer" into the wells.
2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100 µL each of test sample blank (tube-8), test sample and dilutions of standard (tube-1-7) into the appropriate wells.
3) Incubate the precoated plate for 60 minutes at 37°C after covering it with plate lid.
4) Wash the plate with the prepared wash buffer and remove all liquid. *
5) Pipette 100 µL of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
6) Incubate the precoated plate for 30 minutes at 4°C after covering it with plate lid.
7) Wash the plate with the prepared wash buffer and remove all liquid. *
8) Take the required quantity of "6, Chromogen" into a disposable test tube. Then, pipette 100 µL from the test tube into every well. Please do not return the rest of used chromogen in the test tube into "6, Chromogen" bottle in order to avoid contamination.
9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The solution will turn blue.
10) Pipette 100 µL of "7, Stop solution" into the wells. Mix the solution by tapping the side of precoated plate. The solution will turn yellow by addition of "7, Stop solution".
11) Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the solution. Then, run the plate reader and conduct measurement at 450 nm against a reagent blank.
The measurement shall be done within 30 minutes after addition of "7, Stop solution".

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) Test samples should be diluted with "4, EIA buffer", as the need arises.
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) Use only wash buffer contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.

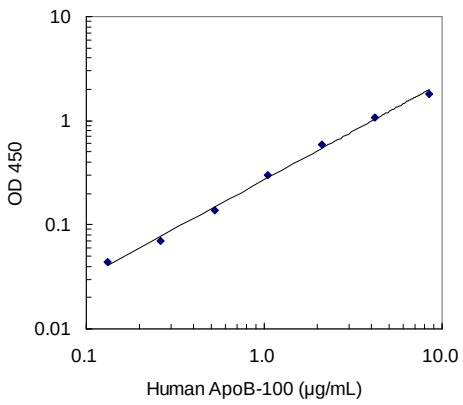
6) Remove the wash buffer completely by tapping the precoated plate on paper towel. Do not wipe wells with paper towel.
7) "6, Chromogen" should be stored in the dark due to its sensitivity against light. Avoid contact of Chromogen with metals.
8) Measurement should be done within 30 minutes after addition of "7, Stop solution".

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

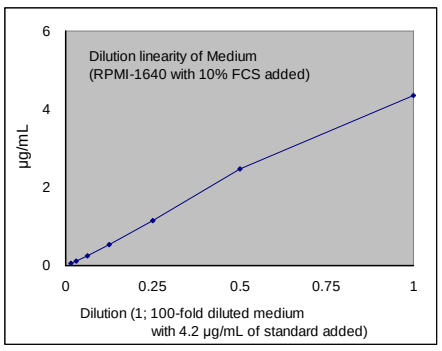
Conc. (µg/mL)	Absorbance (450nm)
8.4	2.968
4.2	1.859
2.1	1.035
1.05	0.565
0.53	0.297
0.26	0.145
0.13	0.077
0 (Test Sample Blank)	0.009

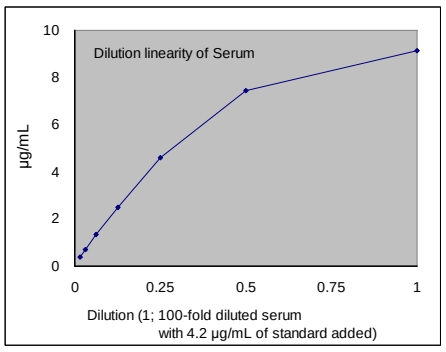


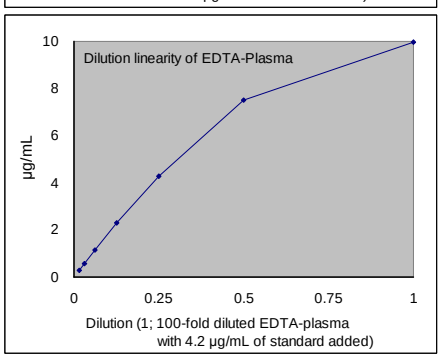
The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

PERFORMANCE CHARACTERISTICS

1. Dilution linearity







2. Added Recovery Assay

Specimen	Theoretical Value (µg/mL)	Measured Value (µg/mL)	%
10%FCS added RPMI-1640 (x2)	4.20	4.57	108.8
	2.10	2.33	111.0
	1.05	1.12	106.7
	0.53	0.54	101.9
	0.26	0.25	96.2
Human Serum (x400)	7.78	7.57	97.3
	5.68	5.88	103.5
	4.63	4.74	102.4
	4.11	4.26	103.6
	3.84	3.88	101.0
Human Plasma (EDTA) (x400)	7.99	7.31	91.5
	5.89	5.47	92.9
	4.84	4.54	93.8
	4.32	3.98	92.1
	4.05	3.80	93.8

3. Intra - Assay

Mean Value (µg/mL)	SD (µg/mL)	CV (%)	n
4.69	0.17	3.6	26
2.62	0.12	4.6	26
0.90	0.05	5.6	26

4. Inter - Assay

Mean Value (µg/mL)	SD (µg/mL)	CV (%)	n
4.82	0.36	7.5	5
2.74	0.20	7.3	5
0.86	0.06	7.0	5

5. Specificity

Substance	Cross-Reactivity
Human ApoB-100	100 %
Human ApoB-48	< 0.1 %

6. Sensitivity

0.03 µg/mL
The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.)

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. Dispose used materials after rinsing them with large quantity of water.
5. Precipitation may occur in "2, Labeled antibody Conc." or "4, EIA buffer" however, there is no problem in the performance.
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.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE AND THE TERM OF VALIDITY

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

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Made in Japan.

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