Human Apo B-48 ELISA KIT
Cat. No. 637-10641

For in-vitro laboratory use only !
Please, read this instruction carefully before use. This is an ELISA (Enzyme Linked ImmunoSorbent Assay) kit for measurement of human apo B-48 with high sensitivity using Sandwich assay principle.

[Advantage]

(1) Rapid assay (total reaction time: 2 hours 50 min).
(2) A small sample volume.
(3) An ecologically excellent preservative is used.
(4) Excellent precision and reproducibility.

[Components]

	Reagents	Amounts
(A)	Anti-apo B-48-coated plate	96 wells (8x12) / 1 plate
(B)	Standard human apo B-48 (Frozen-dried)	128ng/1 vial
(C)	Buffer solution	60ml/1 bottle
(D)	Biotin-conjugated anti-apo B-48 antibody	100µl/ 1 vial
(E)	Peroxidase-conjugated streptavidin	100µl/ 1 vial
(F)	Chromogenic substrate reagent (TMB)	12ml/ 1 bottle
(H)	Reaction stopper (1M H_2SO_4)	12ml/ 1 bottle
(I)	Concentrated washing buffer (10x)	100ml/ 1 bottle

[Assay sample]

Human serum or plasma(heparin or EDTA as anticoagulant) diluted to 100x with buffer (C).

[Purpose of assay]

Quantitative measurement of apolipoprotein B-48

[Assay range]

2.5 – 160 ng/ml

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[Assay operation]

1. Equipments necessary but not included in the kit.

(1) Micropipette (a micropipette able to deliver sample volume with high precision.), and a pipette for repetitive dispensing.
(2) Microplate washing apparatus (a microplate washer or a flashing bottle with nozzle).
(3) A microplate reader (A densitometer for microplate).

2. Preparation of reagents

(1) Washing buffer: Dilute the concentrated washing buffer (I) to 10x with purified water.
(2) Biotin-conjugated anti-apo B-48 (D): Dilute to 100x with the buffer solution(C).
(3) HRP-conjugated streptavidin (E): Dilute to 100x with the buffer solution(C).
(4) Other reagents are used as they are.
(5) All the reagent solutions should be used after getting back to room temperature (20-25C).

3. Assay sample dilution Dilute assay samples to 100x with buffer (C). (An enough stir is necessary.)

4. An example of preparing standard solutions Prepare the original standard solution by adding 400µl of distilled water to the vial (B) and mix well(1800-2200rpm,15-30seconds) to dissolve the content, then prepare a series of standard solutions by a dilution program shown below. After reconstitution, the original standard solution should be used within 24 hours (2-8C). Freezing and thawing of the original standard solution is permitted only once. The use of either polyethylene or polypropylene test tubes is recommended for the dilution prosess. Test tubes of other materials are not suitable due to large absorption.

Concentration (ng/ml)	160	80	40	20	10	5.0	2.5	0
Std. Sol. (µl)	200**	200*	200*	200*	200*	200*	200*	0
Buffer (µl)	200	200	200	200	200	200	200	200

**Original standard solution, *One rank higher standard solution

5. Assay procedure
(1) Remove the cover sheet of the microplate after getting back to room temperature.
(2) Rinse the antibody coated wells (A) by filling the washing buffer and discard 4 times, then strike the plate upside-down onto folded several sheets of paper towel, and remove the excess buffer.
(3) Pipette 50□l of diluted sample into the wells for samples.
(4) Pipette 50□l of the standard solution to the wells for preparing a standard curve.
(5) Shake the plate on a plate shaker at 800-1,000rpm for approximately 5 to 10 seconds.
(6) Incubate for 1 hour at room temperature (20-25C).
(7) Discard the reaction mixture, and then wash wells as described in (2).
(8) Pipette 50□l of biotin-conjugated anti-apo B-48 solution to all wells. Then shake on a plate shaker as (5).
(9) Incubate the plate for 1 hour at room temperature.

(10) Discard the reaction mixture, and then wash the plate as (2).
(11) Pipette 50□l of HRP-conjugated avidin solution to all wells, and shake as (5).
(12) Incubate for 30 minute at room temperature.
(13) Discard the reaction mixture, and wash the plate as (2).
(14) Pipette 50□l of chromogenic substrate solution to wells, and shake as (5).
(15) Let the plate stand for 20 minutes at room temperature.
(16) Add 50 □l of the reaction stopper (H) to all wells and shake as (5).
(17) Measure the absorbance of each well at 450 nm (sub-wave length, 620nm) by a plate reader within 30 minutes.

[Summary of Assay Procedure]

(1) Antibody-coated well plate
↓
(2) Washing 4 times
↓
(3)-(4) Sample (diluted) or Standard 50µl
↓
(5)-(6) Shaking and reaction for 1 hour at room temp .
↓
(7) Washing 4 times
↓
(8) Biotin-conjugated anti-apo B-48 antibody 50µl
↓
(9) Shaking and reaction for 1 hour at room temp.
↓
(10) Washing 4 times
↓
(11) Peroxidase-conjugated avidin 50µl
↓
(12) Shaking and reaction for 30 mins at room temp.
↓
(13) Washing 4 times
↓
(14) Chromogenic substrate solution 50µl
↓
(15) Shaking and reaction for 20 mins. at room temp
↓
(16) Reaction stopper 1M H_2SO_4 50µl
↓
(17) Shaking and measurement of absorbance at 450nm(sub. 620nm)

*Room temp:20-25C

[Calculation of apo B-48 concentration]

(1) Prepare a standard curve using semi-logarithmic or logarithmic section paper by plotting absorbance* (Y-axis) against apo B-48 concentration (ng/ml) on X-axis.
*Absorbance at 450nm minus absorbance at 620nm.

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(2) Using the standard curve, read the apo B-48 concentration of a sample from its absorbance*, and multiply the assay value by dilution rate (in the standard procedure, the dilution rate is 100). Though the assay range is wide enough, in case the absorbance of some samples are higher than that of the highest standard, please repeat the assay after proper dilution (upper limit is 200x) of samples with the buffer solution.
*We recommend the use of 3rd order regression curve or 4-parameter method in computer calculation. Ver.0712

[Important notice in the treatments]

1. Treatment of assay samples

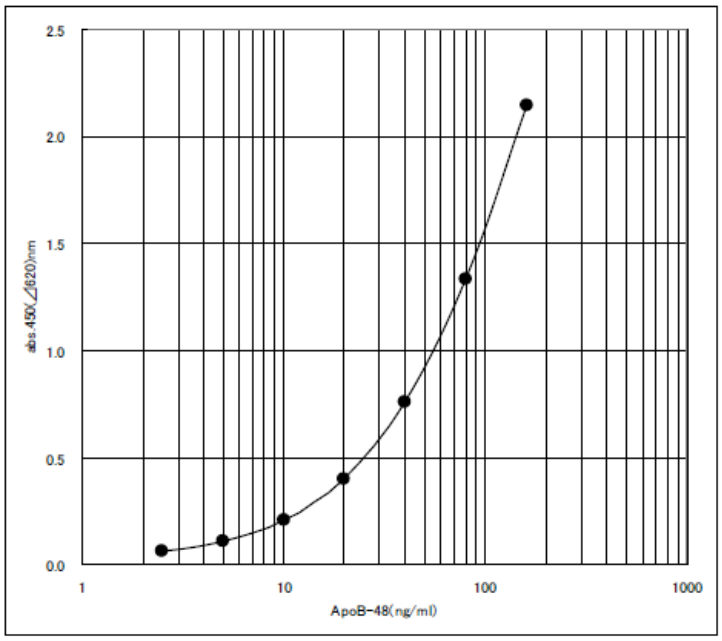
(1) Use serum or plasma samples obtained by ordinary standard method.
(2) Turbid samples or those containing insoluble materials should be centrifuged before assay and remove those materials.
(3) Measure the samples as soon as possible after sampling.
(4) Dilution of assay samples should be made using microtubes before starting assay.

2. Storage of assay samples.

If assay samples have to be stored for a long period, freeze samples and store below –35C. The original standard solution should be prepared immediately before assay. But if you have to store it, store it below –35C. Avoid repeated freezing and thawing for both samples and the standard solution.

[Assay range and assay validation]

1. A model standard curve

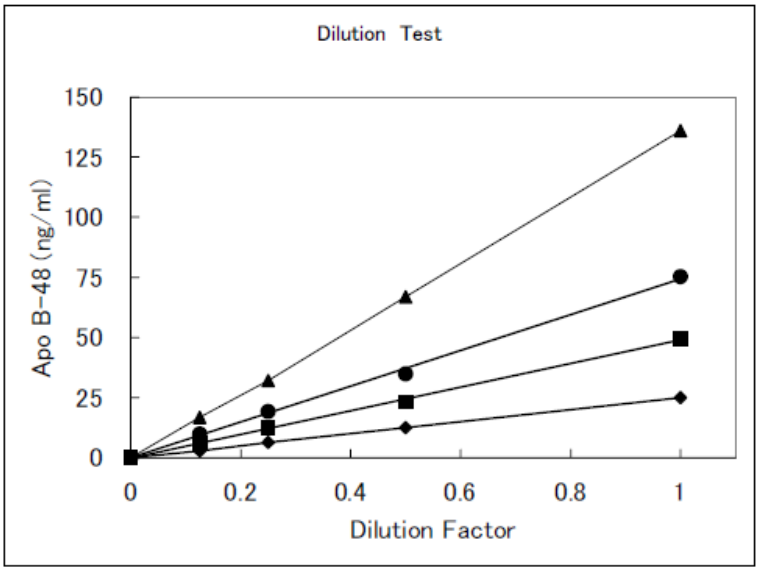


2. Specificity

Cross-reaction to human apo B-100 is less than lower detection limit.
3. Precision and reproducibility
(1) Within assay variation (3 samples, 5 replicates assay) Average C.V. is 3.5%.
(2) Reproducibility (3 samples, triplicates assay, 3 days) Average C.V. is 2.8 – 8.6% Ver.0712
(3) Recovery test

Sample	Added (ng/ml)	Found (ng/ml)	Recovered (ng/ml)	Recovery (%)
1	0	31.3	–	–
	20.0	51.2	19.9	99.5
	40.0	70.1	38.8	97.0
	60.0	87.7	56.4	94.0
2	0	4.8	–	–
	5.0	10.0	5.2	104
	10.0	15.3	10.5	105
	15.0	19.7	14.9	100

4. Dilution test



5. Assay data of Apolipoprotein B-48

Sample: Normal human plasma before meal Duplicate assay, Unit: μg/ml

Sample No.	Assay Value	Sample No.	Assay Value	Sample No.	Assay Value
1	3.59	7	4.16	13	2.88
2	5.66	8	8.12	14	5.28
3	5.86	9	5.58	15	3.05
4	6.19	10	2.69	16	2.65
5	6.56	11	2.83	17	4.69
6	4.63	12	4.11	18	4.23

mean	4.60	SD	1.54

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Important notes to minimize assay variation in Human Apo B-48 assay

Temperature
Every reagent should be put back to 20 to 25C before assay. This is attained by keeping the reagents for about one hour on a laboratory table before starting assay if the room temperature is 22C. As a rule, dilution of the reagents, preparation of the standard solutions and assay samples should be made immediately before assay.

Dissolving the original standard preparation
The original frozen-dried standard preparation should be dissolved by adding the indicated volume of purified water and enough vortex-stirring (1800~2200rpm, 10 seconds x 3 times) with a good care not to avoid bubble formation. Bubbles may cause denaturation of the protein. Be sure that the material is completely dissolved to give clear solution.

Caution about vortex-stirring:
Vortexing should be started from the resting state. Do not put a tube on a rotating vortex, because this often causes bubble formation.

Preparation of standard solutions, and dilution of assay samples
Use duly calibrated micropipettes. Precise pipetting is essential for the excellent precision. At every step of serial dilution, enough stirring is necessary using vortex-type stirrer (1800~2200rpm, 10 seconds x 3 times). The buffer contains a detergent, so be careful not to make bubbles while stirring.

Delivering of samples and reagents to wells
In the pipetting of samples and reagents to assay wells, be careful not to make bubbles. Bubbles may cause variation of the assay results. For the delivery of reagents, we recommend the use of multiple dispenser, like Eppendorf's Multipette Plus. After delivery of a reagent, never forget to stir the assay plate on a plate shaker. Adjust the rotation speed of the shaker to give homogeneity of the mixture.

Reaction period
The counting of the reaction period is usually started from the pipetting of the first well, however, if the pipetting takes a long time (more than 15 minutes), start counting from the last pipetting. The use of a multi-pipette or a repeating delivering apparatus is also recommended.

Reaction temperature
Keep the reaction temperature between 20~25C. We recommend the use of an incubator set at this temperature range.

Washing of the plate
When a plate washer is used, this apparatus sometimes requests the adjustment of the pressure or speed of the addition and aspiration of the washing solution. Mismatching of these conditions of the apparatus often.

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