

Human Apolipoprotein B ELISA Kit

Strip well format. Reagents for up to 96 tests.

Catalog No: CKH019A

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Size: 1 x 96 wells

Size: 5 x 96 wells

Introduction:

The human Apolipoprotein B (ApoB) antigen assay is intended for the quantitative determination of total ApoB in human plasma, serum, urine & other biological fluids.

ApoB is the primary protein constituent of low density lipoprotein, very-low density lipoprotein, and chylomicrons. ApoB directs cholesterol and triglyceride containing particles to tissues by ApoB receptor binding and internalization. The ratio of ApoB to Apolipoprotein A1 (ApoA1), the major protein component of high density lipoprotein, is an effective predictor of cardiovascular disease.

Principle of the Assay:

Human ApoB will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, monoclonal anti-human ApoB primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody is reacted with the peroxidase conjugated secondary antibody. Following an additional washing step, TMB substrate is used for color development at 450 nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of human ApoB. The amount of color development is proportional to the concentration of total ApoB antigen in the sample.

Reagents and materials supplied with the kit:

Items	Quantity
A. 96-well Strip Plate (8 x 12 wells) coated with capture antibody, blocked, and dried	1 x 96 wells (8 x 12-well removable strips)
B. 10x Wash Buffer Concentrate	1 bottle (50 ml)
C. 10x Diluent	1 bottle (50 ml)
D. Human ApoB Standard:	1 vial (lyophilized)
D. Anti-Human ApoB Primary Monoclonal Antibody	1 vial (lyophilized)
E. HRP conjugated Anti-Mouse Secondary Antibody	1 vial
F. TMB Substrate Solution	1 bottle (10 ml)

*Hazard Information:

Avoid skin and eye contact when using TMB substrate solution as it may be irritating to eyes, skin and respiratory system. Wear safety goggles and gloves.



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Storage of Kit Reagents:

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standard may be stored at -80°C for later use. **Do not freeze-thaw the standard more than once.** Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

Materials/Reagents required but not provided:

- 1-channel pipettes covering 0-10 µl and 200-1000 µl
- 12-channel pipette for 30-300 µl
- Paper towels or Kimwipes
- Polypropylene tubes for dilution of standard
- 50 ml tubes
- 1 N H₂SO₄
- De-ionized (DI) or distilled water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- TBS and Blocking Buffers
- Microtiter plate spectrophotometer operable at 450 nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm

Precautions:

- **DO NOT** mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- **DO NOT** pipette reagents by mouth.
- Always pour TMB substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- **DO NOT** pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

Preparation of Buffers, Specimens, and Standards:

TBS Buffer

0.1 M Tris + 0.15 M NaCl, pH 7.4

Blocking Buffer

3% BSA in TBS Buffer

Diluent

Dilute 50 ml of 10X diluent concentrate with 450 ml of deionized water.

Wash Buffer

Dilute 50 ml of 10X wash buffer concentrate with 450 ml of deionized water.



Specimen Collection

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Assay Procedure

Perform assay at room temperature. Vigorously shake plate (300 rpm) at each step of the assay.

Preparation of Standard

Reconstitute standard by adding 1 ml of 1X Diluent directly to the vial and agitate to completely dissolve contents. This will result in a 5,000 ng/ml standard solution.

Table 1: Dilution table for preparation of Human ApoB Standard:

ApoB concentration (ng/ml)	Dilutions
5,000	Straight from the vial
2,000	600 μl Diluent + 400 μl (5,000 ng/ml)
1,000	500 μl Diluent + 500 μl (2,000 ng/ml)
500	500 μl Diluent + 500 μl (1,000 ng/ml)
200	600 μl Diluent + 400 μl (500 ng/ml)
100	500 μl Diluent + 500 μl (200 ng/ml)
50	500 μl Diluent + 500 μl (100 ng/ml)
20	600 μl Diluent + 400 μl (50 ng/ml)
10	500 μl Diluent + 500 μl (20 ng/ml)
5	500 μl Diluent + 500 μl (10 ng/ml)
0	500 μl Diluent Zero point to determine background

NOTE: Dilutions for the Standard Curve and zero standard must be made and applied to the plate immediately.

Standard and Unknown Addition:

1. Remove microtiter plate from bag.
2. Add 100 μl of ApoB standards (in duplicate) and unknowns to wells. Carefully record the position of standards and unknowns.
3. Shake plate at 300 rpm for 30 minutes.
4. Wash wells three times with 300 μl wash buffer.
5. Remove excess wash by gently tapping plate on paper towel or Kimwipe.

NOTE: The assay measures ApoB antigen in the 5-5,000 ng/ml range. Samples giving human ApoB levels above 5,000 ng/ml should be diluted in diluent before use. A 1:20,000 to 1:80,000 dilution for normal human plasma is suggested for best results.



Primary Antibody Addition:

6. Reconstitute primary antibody by adding 10 ml blocking buffer to vial.
7. Agitate gently to completely dissolve contents.
8. Add 100 μ l to all wells.
9. Shake plate at 300 rpm for 30 minutes.
10. Wash wells three times with 300 μ l wash buffer.
11. Remove excess wash by gently tapping plate on paper towel or Kimwipe.

Secondary Antibody Addition:

12. Briefly centrifuge vial before opening.
13. Dilute 2 μ l conjugated secondary antibody in 10 ml BSA blocking buffer.
14. Add 100 μ l to all wells.
15. Shake plate at 300 rpm for 30 minutes.
16. Wash wells three times with 300 μ l wash buffer.
17. Remove excess wash by gently tapping plate on paper towel or Kimwipe.

Substrate Incubation:

18. Add 100 μ l TMB Substrate Solution to all wells and shake plate for 1-5 minutes. Substrate will change from colorless to different strengths of blue.
19. Stop reaction by adding 50 μ l of 1 N H₂SO₄ Stop Solution to all wells when samples are visually in the same range as the standards. Add Stop Solution to wells in the same order as substrate at which time the color will change from blue to yellow.
20. Mix thoroughly and read final absorbance values at 450 nm. For best results read plate immediately.

Measurement:

21. Set the absorbance at 450 nm in a microtiter plate spectrophotometer.
22. Measure the absorbance in all wells at 450 nm. Subtract zero point from standards and unknowns to determine corrected absorbance (A_{450}).

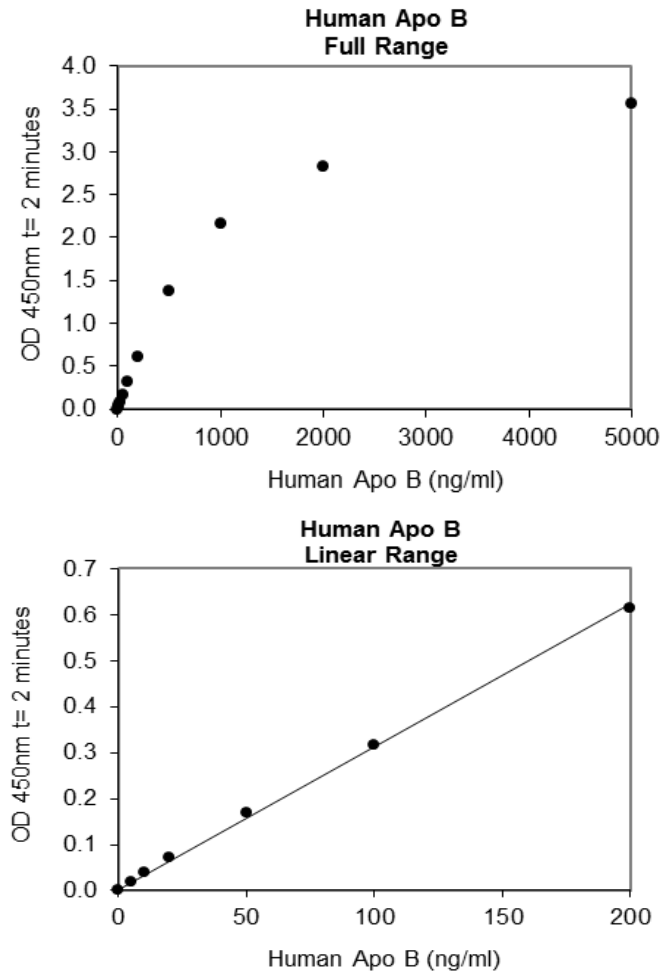
Assay Calibration:

23. Plot A_{450} against the amount of ApoB in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve.
24. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of ApoB in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.



Typical Standard Curves:

These standard curves are examples only. Standard curves must be run with each assay.



Expected Values:

ApoB is present in human plasma and serum at a concentration of 0.5-1.25 mg/ml in adult males, 0.45-1.2 mg/ml in adult females, 0.11-0.31 mg/ml in newborns, and 0.23-1.39 mg/ml in children [2]. The ratio of ApoA1/ApoB ranges from 0.85-2.24 in males and 0.76-3.23 in females.



Performance Characteristics:**Sensitivity:**

The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD₄₅₀: 0.076-0.096) and calculating the corresponding concentration. The MDD was 4.9 ng/ml.

Sample Values:

Samples were evaluated for the presence of the antigen at varying dilutions.

Sample Type	Dilution	Mean
Citrate Plasma	1:20,000	2.65 mg/ml
	1:40,000	2.85 mg/ml
	1:80,000	2.85 mg/ml
Milk	Undiluted	1340 ng/ml
Urine	Undiluted	10 ng/ml

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