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Human Apolipoprotein B ELISA Kit

Catalog # HAPOBKT

Strip well format. Reagents for up to 96 tests.

Rev: May 2015

INTENDED USE

This human Apolipoprotein B (ApoB) antigen assay is intended for the quantitative determination of total ApoB in human plasma, serum, urine & other biological fluids. **For research use only.**

BACKGROUND

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 [1].

ASSAY PRINCIPLE

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 450nm. 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 PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-human ApoB antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 30ml
- **10X Diluent:** 1 bottle of 50ml
- **Human ApoB standard:** 1 vial lyophilized standard
- **Anti-human ApoB primary antibody:** 1 vial lyophilized monoclonal antibody
- **Anti-mouse horseradish peroxidase secondary antibody:** 1 vial concentrated HRP labeled antibody
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY

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.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- 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.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- 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 REAGENTS

- **TBS buffer:** 0.1M Tris, 0.15M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **1X Diluent:** Dilute 30ml of 10X diluent concentrate with 270ml of deionized water.
- **1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water.

SAMPLE COLLECTION

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

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

Preparation of Standard

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

Dilution table for preparation of human ApoB standard:

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

Remove microtiter plate from bag and add 100µl ApoB standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. 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

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Secondary Antibody Addition

Briefly centrifuge vial before opening. Dilute 2µl of conjugated secondary antibody in 10ml of blocking buffer and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation

Add 100µl TMB substrate to all wells and shake plate for 1-5 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 or HCl 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 upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

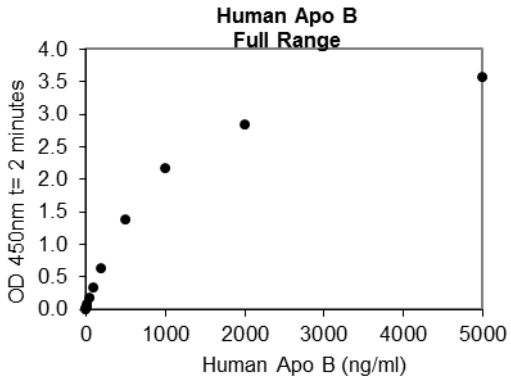
Measurement

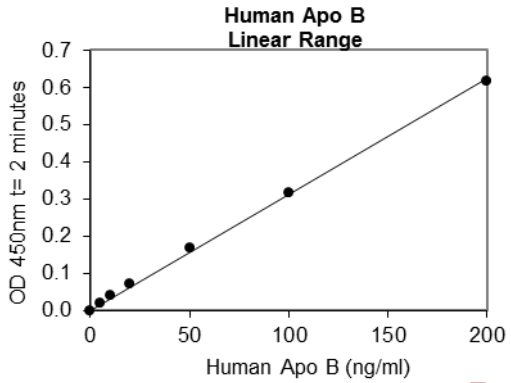
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results

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. 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.

A typical standard curve (EXAMPLE ONLY):





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 OD_{450}: 0.076-0.085) and calculating the corresponding concentration. The MDD was 1.5 ng/ml.

Intra-assay Precision: These studies are currently in progress. Please contact us for more information.

Inter-assay Precision: These studies are currently in progress. Please contact us for more information.

Recovery: These studies are currently in progress. Please contact us for more information.

Linearity: These studies are currently in progress. Please contact us for more information.

Specificity: These studies are currently in progress. Please contact us for more information.

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

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

REFERENCES

1. Lu M *et al.*: J Biomed Res. 2011, 25(4):266-73.
2. Pagana KD and Pagana TJ: Mosby's Diagnostic and Laboratory Test Reference, 11th Edition. 2012.

Example of ELISA Plate Layout
96 Well Plate: 22 Standard wells, 74 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	200 ng/ml	500 ng/ml	1,000 ng/ml	2,000 ng/ml	5,000 ng/ml	
B	0	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	200 ng/ml	500 ng/ml	1,000 ng/ml	2,000 ng/ml	5,000 ng/ml	
C												
D												
E												
F												
G												
H												