

**INTENDED USE**

This human apolipoprotein C-III (ApoC3) antigen assay is intended for the quantitative determination of total ApoC3 in human plasma, serum, urine & other biological fluids. **For research use only.**

**BACKGROUND**

Human ApoC3 is a 79 amino acid peptide synthesized in the liver and intestine and bound to the surface of triglyceride rich particles such as very low-density lipoprotein and chylomicrons in plasma [1]. ApoC3 controls the triglyceride composition of these particles by inhibiting lipoprotein lipase [2]. ApoC3 loss-of-function mutations have been associated with markedly reduced levels of triglycerides and remnant cholesterol and a decrease in coronary-artery calcification, a surrogate marker for atherosclerosis [3]. Therefore, ApoC3 is a potential new target for reducing residual cardiovascular risk.

**ASSAY PRINCIPLE**

Human ApoC3 will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, biotinylated primary antibody binds to the captured protein. Excess primary antibody is washed away, and bound antibody is reacted with horseradish peroxidase conjugated streptavidin. 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 ApoC3. The amount of color development is proportional to the concentration of total ApoC3 antigen in the sample.

**REAGENTS PROVIDED**

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-human ApoC3 antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Human ApoC3 standard:** 1 vial lyophilized standard
- **Anti-human ApoC3 primary antibody:** 1 vial lyophilized polyclonal antibody
- **Horseradish peroxidase-conjugated streptavidin:** 1 vial concentrated HRP labeled streptavidin
- **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 and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody 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<sub>2</sub>SO<sub>4</sub> 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 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  $\leq -20^{\circ}\text{C}$ . 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 according to the C of A.** Agitate gently to completely dissolve contents.

Dilution table for preparation of human ApoC3 Standard:

ApoC3 concentration (ng/ml)	Dilutions
5	950 $\mu\text{l}$ (BB) + 50 $\mu\text{l}$ (from vial)
2	600 $\mu\text{l}$ (BB) + 400 $\mu\text{l}$ (5ng/ml)
1	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (2ng/ml)
0.5	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (1ng/ml)
0.2	600 $\mu\text{l}$ (BB) + 400 $\mu\text{l}$ (0.5ng/ml)
0.1	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (0.2ng/ml)
0.05	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (0.1ng/ml)
0.02	600 $\mu\text{l}$ (BB) + 400 $\mu\text{l}$ (0.05ng/ml)
0.01	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (0.02ng/ml)
0	500 $\mu\text{l}$ (BB) 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 100 $\mu\text{l}$  ApoC3 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 300 $\mu\text{l}$  wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures ApoC3 antigen in the 0.01-5 ng/ml range. Samples giving human ApoC3 levels above 5 ng/ml should be diluted in blocking buffer before use. A 1:200,000-1:1,000,000 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 100 $\mu\text{l}$  to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 $\mu\text{l}$  wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

### **Streptavidin-HRP Addition**

Briefly centrifuge vial before opening. **See C of A for lot specific dilution instructions.** Add 100 $\mu\text{l}$  of your dilution to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 $\mu\text{l}$  wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

### **Substrate Incubation**

Add 100 $\mu\text{l}$  TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50 $\mu\text{l}$  of 1N  $\text{H}_2\text{SO}_4$  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.

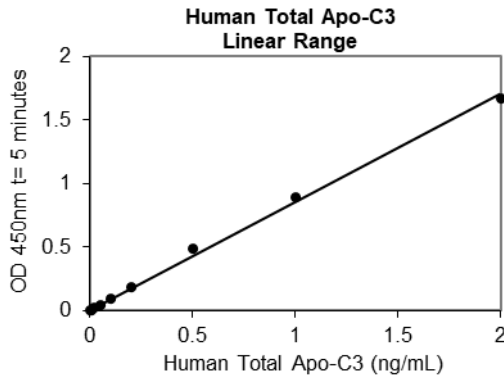
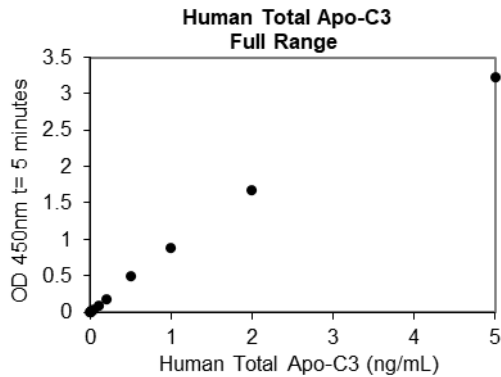
### **Measurement**

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 ApoC3 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 ApoA1 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**

The average concentration of ApoC3 in normal human plasma is 125 µg/ml [4].

**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 and calculating the corresponding concentration. **See C of A for lot specifications.**

**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
ACD Plasma	1:200,000	144 µg/ml
	1:400,000	132 µg/ml
	1:800,000	140 µg/ml

**DISCLAIMER**

This information is believed to be correct but does not claim to be all-inclusive and should 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**

- Galton DJ: Curr Opin Lipidol. 2017, 28:308-312.
- Brown WV and Baginsky ML: Biochem Biophys Res Commun. 1972, 46:375-82.
- Jørgensen AB *et al.*: N Engl J Med. 2014, 371:32-41.
- Qamar A *et al.*: Arterioscler Thromb Vasc Biol. 2015, 35:1880-1888.

**Example of ELISA Plate Layout**

**96 Well Plate: 20 Standard wells, 76 Sample wells**

	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>
<b>A</b>	0	0.01 ng/ml	0.02 ng/ml	0.05 ng/ml	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml		
<b>B</b>	0	0.01 ng/ml	0.02 ng/ml	0.05 ng/ml	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml		
<b>C</b>												
<b>D</b>												
<b>E</b>												
<b>F</b>												
<b>G</b>												
<b>H</b>												