cellsciences.com

# Human Apolipoprotein A1 ELISA Kit

Strip well format. Reagents for up to 96 tests.

**Catalog No:** CKH018A **Size:** 1 x 96 wells
**Catalog No:** CKH018B **Size:** 5 x 96 wells

**Introduction:**
The human Apolipoprotein A (ApoA1) antigen assay is intended for the quantitative determination of total ApoA1 in human plasma, serum, urine & other biological fluids.

ApoA1 is the major protein constituent of high density lipoprotein which is the densest of the lipoprotein aggregates. ApoA1 participates in the reverse transport of cholesterol from tissues to the liver and is a cofactor for lecithin cholesterol acyl transferase. The ratio of ApoA1 to Apolipoprotein B (ApoB), the primary component of low density lipoprotein, is an effective predictor of cardiovascular disease.

**Principle of the Assay:**

Human ApoA1 will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, monoclonal anti-human ApoA1 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 ApoA1. The amount of color development is proportional to the concentration of total ApoA1 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 ApoA1 Standard: | 1 vial (lyophilized) |
| D. Anti-Human ApoA1 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.

**Cell Sciences**®
480 Neponset Street
Bldg 12A
Canton, MA 02021

Toll Free: 888-769-1246
Phone: 781-828-0610
Fax: 781-828-0542

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

**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 $H_2SO_4$
- 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 ≤ -20°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 ApoA1 Standard:**

| ApoA1 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<br>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 ApoA1 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 ApoA1 antigen in the 5-5,000 ng/ml range. Samples giving human ApoA1 levels above 5,000 ng/ml should be diluted in diluent before use. A 1:40,000 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 µl to all wells.
9. Shake plate at 300 rpm for 30 minutes.
10. Wash wells three times with 300 µl 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 µl conjugated secondary antibody in 10 ml BSA blocking buffer.
14. Add 100 µl to all wells.
15. Shake plate at 300 rpm for 30 minutes.
16. Wash wells three times with 300 µl wash buffer.
17. Remove excess wash by gently tapping plate on paper towel or Kimwipe.

**Substrate Incubation:**

18. Add 100 µl 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 µl of 1 N $H_2SO_4$ 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:**

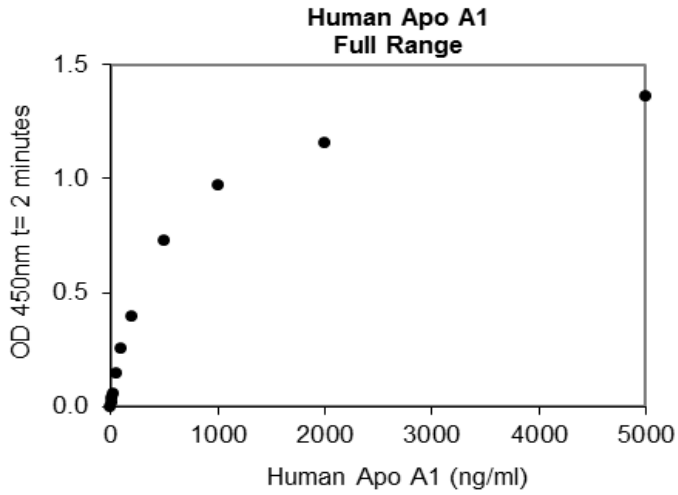
21. Set the absorbance at 450 nm in a microtiter plate spectrophotometer.
22. Measure the absorbance in all wells at 450 nm. Subtract zero point from standards and unknowns to determine corrected absorbance ($A_{450}$).

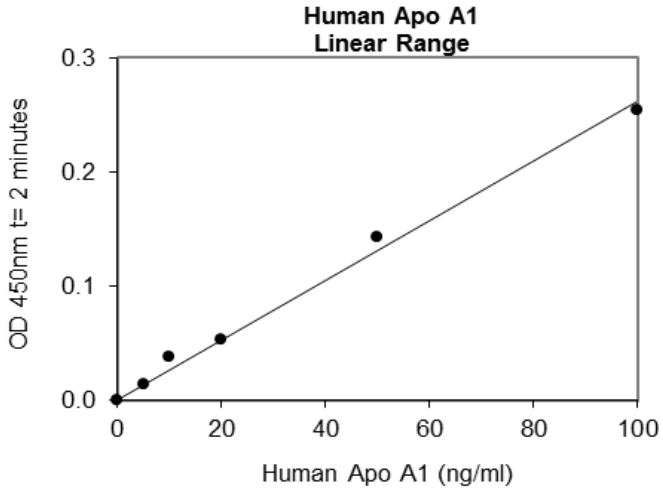
**Assay Calibration:**

23. Plot $A_{450}$ against the amount of ApoA1 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 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.

**Typical Standard Curves:**

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




**Expected Values:**

ApoA1 is present in human plasma and serum at a concentration of 0.75-1.6 mg/ml in adult males, 0.8-1.75 mg/ml in adult females, 0.38-1.06 mg/ml in newborns, and 0.6-1.67 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.

**Cell Sciences** ®
480 Neponset Street
Bldg 12A
Canton, MA 02021

Toll Free: 888-769-1246
Phone: 781-828-0610
Fax: 781-828-0542

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

**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 $OD_{450}$: 0.076-0.096) 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 | 1:40,000 | 2.079 mg/ml |
| | 1:80,000 | 2.016 mg/ml |
| Milk | Undiluted | 564 ng/ml |
| Urine | Undiluted | Undetectable |

**NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.**

**Cell Sciences** ®
480 Neponset Street
Bldg 12A
Canton, MA 02021

Toll Free: 888-769-1246
Phone: 781-828-0610
Fax: 781-828-0542

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com