Apolipoprotein A1 (Apo A1) ELISA

Catalog Number: AA131-K01
1 x 96 Wells
For Research Use Only (RUO). Not for use in clinical, diagnostic or therapeutic procedures.
v. 1.0
Intended Use:

The Eagle Biosciences Apolipoprotein A1 (Apo A1) ELISA Assay Kit is intended for the quantitative determination of total apolipoprotein A1 in serum and plasma by enzyme linked immunoassay (ELISA). The Apolipoprotein A1 (Apo A1) ELISA assay kit is for research use only and not to be used in diagnostic procedures.

Assay Background:

Apo A1 is primarily found in high density lipoprotein (HDL) particle. It serves the function of preventing the accumulation of cholesterol loaded macrophages which deposit on the arterial wall as foam cells. This is the prominent early feature of atherosclerotic lesion formation ultimately resulting in atherosclerosis. Apo A1 is a single polypeptide with a molecular weight of 28 Kd. Its primary function is to activate LCAT within the HDL complex, which catalyzes the esterification of cholesterol. This results in a more soluble cholesterol–HDL complex which increases the cholesterol transport capacity of the HDL particle for subsequent removal by the liver. Apo A1 is therefore a convenient marker for assessing the cholesterol clearing capacity of the blood, and studies have clearly indicated that it is a better discriminator of angiographically documented coronary artery disease than HDL cholesterol.

Principle of Procedure:

The Apolipoprotein A1 (Apo A1) ELISA assay kit determines human apolipoprotein A1 according to the “sandwich” principle. Apolipoprotein A1 in samples and standards binds to antibodies which are coated to the microtiter plate. After a washing step a peroxidase labeled detection antibody is added. A second washing step is followed by the addition of the substrate which is converted to a colored product by the peroxidase. The reaction is terminated by the addition of an acidic stop solution. The optical densities are read at 450 nm in a microtiter plate reader. The apolipoprotein A1 concentration can be calculated from the standard curve.

Materials Provided:

The expiration date for the Apolipoprotein A1 (Apo A1) ELISA assay kit and each component is stated on the label(s). Store all components at 2-8°C with the exception of the Apo A1 standard, which should be stored at -20°C.
- Anti-human Apo A1 coated microwell strips - 12x8 with plastic frame
- HRP conjugated affinity purified goat anti-Apo A1 – 12 mL
- Apo A1 standard** (pre-diluted 1:10,000) – 1 mL (store at -20°C)
- TMB/Peroxide Substrate color developer III – 12 mL
- Stop Solution: Sulfuric acid termination reagent (0.5 N) – 12 mL
- 15X wash buffer concentrate – 2x60 mL

**Note: This Apo A1 Standard has been calibrated against the International Federation of Clinical Chemistry (IFCC) Standard, lot # 293, and has been demonstrated to recover 100% of this standard.

**Reagent and Sample Preparation:**

- Dilute the 15X wash buffer provided in the Apolipoprotein A1 (Apo A1) ELISA assay kit 1:15 using one part wash buffer concentrate and 14 parts reagent grade water.
- Dilute each serum or plasma specimen to be tested 1:10,000 with diluted wash buffer.
- Prepare serial two-fold dilutions of the human Apo A1 standard (neat, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64) with the diluted wash buffer. Use the diluted wash buffer alone in the blank control well.

**Assay Procedure:**

Allow each reagent of the Apolipoprotein A1 (Apo A1) ELISA assay kit to reach room temperature before use.

1. Add 100 µL of diluted specimen or standard to each microwell.
2. Incubate at room temperature for 2 hours.
3. Decant and wash each microwell four times with diluted wash buffer (Dilute wash buffer 1:15 with reagent grade water).
4. Add 100 µL of HRP conjugated goat Apo-A1 to each well.
5. Incubate at room temperature for 2 hours.
6. Decant and wash as in step 3.
7. Add 100 µL of TMB/peroxide substrate and incubate at room temperature for 30 minutes.
8. Terminate the reaction with 100 µL of Stop Solution 0.5N sulfuric acid.
9. Zero the microwell reader at 450 nm using the blank control well.
10. Construct a standard curve using the O.D. values obtained for each of the standards.

**Calculations:**

For calculating the results we recommend using the 4-parameter algorithm. If this algorithm is not available a “point to point” or a “spline” function can be used. Manual processing of results: Correct each absorbance value by subtracting the background absorbance (blank). Estimate the mean value for each duplicate. Construct a standard curve by plotting the mean absorbance value for each standard (y-axis) against the corresponding concentration (x-axis) on semi-log graph paper and read the concentration of unknowns off the curve.

The curve given above is only for demonstration. It must not be used for calculation of your samples.
**Dynamic Range:**

The dynamic range of the Apolipoprotein A1 (Apo A1) ELISA assay kit is 0.52 µg/dL – 33.3 µg/dL.

**Reproducibility:**

The Apolipoprotein A1 (Apo A1) ELISA assay kit was found to have a reproducibility with a C.V. 6%-10% depending on region of the standard curve.

**Warranty Information**

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