Apolipoprotein B (Apo B) Total ELISA

Catalog Number: APB31-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 B (Apo B) ELISA assay kit is intended for the quantitative determination of total Apolipoprotein B in serum and plasma by enzyme linked immunoassay (ELISA). The Apolipoprotein B (Apo B) ELISA assay kit is for research use only and not to be used in diagnostic procedures.

**Assay Background:**

Over 90% of low density lipoprotein (LDL) particle is composed of Apo B. It serves the function of solubilizing cholesterol within the LDL complex, which in turn increases the transport capacity of LDL for subsequent deposit on the arterial wall. Apo B is therefore a convenient marker for assessing the cholesterol depositing capacity of the blood, and studies have clearly indicated it as a better discriminator of angiographically documented coronary artery disease than LDL cholesterol.

**Principle of Procedure:**

The Apolipoprotein B (Apo B) ELISA assay kit determines total human Apolipoprotein B according to the “sandwich” principle. Apolipoprotein B 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 B concentration can be calculated from the standard curve.

**Materials Provided:**

The expiration date for the Apolipoprotein B (Apo B) 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 B standard, which should be stored at -20 °C.

- Anti-Human Apo B coated microwell strips 12x8 with plastic frame
- HRP conjugated affinity purified goat anti-Apo B -12mL
- Apo B standard** (pre-diluted 1:1000)
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- TMB/peroxide substrate color developer – 12mL
- Apo B specimen diluent – 60mL
- Sulfuric acid termination reagent (0.5N) – 12mL
- 15X Wash buffer concentrate – 60mL

**Note:** This Apo B 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 1:15 using one part wash buffer concentrate and 14 parts reagent grade water.

- Dilute each serum or plasma specimen to be tested 1:1000 by first diluting 1:100 in Phosphate buffered saline (PBS), and then making a subsequent 1:10 dilution with the Apo B specimen diluent provided, to form a final dilution of 1:1000.

- Prepare serial two fold dilutions of the human Apo B standard: Neat, 1:2, 1:4, 1:8 etc. with the specimen diluent provided. Use the specimen diluent alone as the blank control well.

**Assay Procedure:**

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

1. Add 100µL of diluted specimen, blank, or standard to each microwell
2. Incubate at room temperature for 45 minutes
3. Decant and wash each microwell five times with wash buffer (dilute buffer 1:15 with reagent grade water)
4. Add 100 µL of HRP conjugated goat anti-Apo B to each well
5. Incubate at room temperature for 45 minutes
6. Decant and wash as in step 3
7. Add 100 µL of TMB/peroxide substrate and incubate at room temperature for 15 minutes
8. Terminate the reaction with 100 µL of 0.5N sulfuric acid

Calculations:

For calculating the results we recommend using the 4-parameter algorithm. First, zero the microwell reader at 450 nm using the blank control well. 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.

*Interpolated concentrations greater than 150 mg/dL should be sub diluted 1:4 and re-assayed then corrected mathematically*

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 B (Apo B) ELISA assay kit is 2.0 µg/dL – 259 µg/dL.

Reproducibility:

The Apolipoprotein B (Apo B) 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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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.