Apolipoprotein E (Apo E) Total ELISA

Catalog Number: APE31-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 E (Apo E) ELISA assay kit is intended for the quantitative determination of total apolipoprotein E in serum and plasma by enzyme linked immunoassay (ELISA). The Apolipoprotein E (Apo E) ELISA assay kit is for research use only and not to be used in diagnostic procedures.

Assay Background:

Apolipoprotein E (Apo E) is a member of the apolipoprotein family, which carries proteins that combine with lipids, to form lipoprotein molecules. This particular protein is 299 amino acids long, with a molecular weight of 34 kD. Apo E is essential for the removal of excess cholesterol from the blood and transporting it to the liver for further processing. Thus; Apo E determines the homeostasis of cholesterol and triglycerides.

Apo E has been recognized for its important roles in lipoprotein metabolism, cardiovascular disease, Alzheimer’s disease, aging, and many other biological functions. Apo E plasma concentration levels in healthy individuals may vary with respect to different biological functions. Normal Apo E serum levels are approximately 5-8 mg/dL. Sex, age, and lifestyle contribute to trends of concentrations. Hyperlipidemia is associated with very high Apo E levels or defective/deficient Apo E protein. Normal Apo E levels are compatible with complete plasma cholesterol homeostasis.

Plasma concentration of Apo E in association with Apo E genotype, may yield useful laboratory information. The Apo E gene is polymorphic, with three common alleles (E2, E3, E4). These isoforms differ in a single amino acid substitution and in their affinity for LDL-receptors. Apo E2 allele is associated with lower plasma cholesterol concentrations with the E4 allele is associated with higher levels. E4 has also been suggested to increase an individual’s risk for developing Type 2 Alzheimer’s disease. The Apo E genotype is also a determinant risk for certain diseases such as cardiovascular disease.

There is a possible epigenetic role regarding gene-environment interactions. The relationship between Apo E genotype and plasma concentrations may be significantly influenced by age, sex, and body weight distribution.
Principle of Procedure:

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

Materials Provided:

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

- Anti-human Apo E coated microwell strips - 12x8 with plastic frame
- HRP conjugated affinity purified goat anti-Apo E – 12 mL
- **Apo E standard (pre-diluted 1:500) – 1 mL
- TMB/Peroxide substrate color developer III – 12 mL
- Sulfuric acid termination reagent (0.5 N) – 12 mL
- 15X wash buffer concentrate – 2x60 mL

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 1:500 with diluted wash buffer.

- Prepare serial two-fold dilutions of the human Apo E 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 E (Apo E) 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 1 hour.
3. Decant and wash each microwell five times with diluted wash buffer (Dilute wash buffer 1:15 with reagent grade water).
4. Add 100 µL of HRP conjugated goat anti-human Apo E to each well.
5. Incubate at room temperature for 1 hour.
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.
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 E (Apo E) ELISA assay kit is 0.4 - 26µg/dL.

**Reproducibility:**

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

**References:**


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