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Total Human Apolipoprotein B (APO B) ELISA Assay

REF

Catalog No. E4-242

IVD

For Research Use Only

Intended Use:

To quantitate total human Apolipoprotein B (Apo B)

Principle of Procedure:

Solid phase capture sandwich ELISA assay using a microwell format.

Shelf Life:

The expiration date for the package and each component is stated on the label(s). Store all components at 2°-8° degrees C with the exception of the standard, which should be stored at -20°C .

Patient and Standard Dilutions:

Dilute each serum, plasma or tissue culture fluid specimen to be tested **1:1,000 by first diluting 1:100** in PBS and then making a subsequent 1:10 dilution with the Apo B specimen diluent provided to form a final dilution of 1:1,000. Prepare serial two fold dilutions of the human Apo B standard: Neat (N), 1:2, 1:4, 1:8 etc. with the specimen diluent provided. Use the specimen diluent alone as the blank control well.

Materials Supplied:

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)- 1 ml
TMB/peroxide substrate color developer –12mL
Apo B specimen diluent – 60mL
Sulfuric acid termination reagent (0.5N) –12mL
15 X Wash buffer concentrate –60mL

Limitations of the Procedure:

No single assay should be used as the only basis for arriving at a diagnostic conclusion.

Dynamic Range:
2.11 mg/dL – 264mg/dL

Reproducibility:
C.V. 6%-10% depending upon the region of the standard curve.

Assay Procedure:

* Allow each reagent to reach room temperature before use
1. Add 100uL of *diluted* specimen 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 100uL 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 100uL of TMB/peroxide substrate and incubate at room temperature for 15 minutes
8. Terminate the reaction with 100uL of 0.18N sulfuric acid
9. Zero the microwell reader at 450nm using the specimen diluent zero control well
10. Determine the optical density (O.D.) of the remaining wells
11. Construct a standard curve using the O.D. values obtained for each of the standards
12. Interpolate the unknowns from the standard curve

Note: This Apo B Standard has been calibrated against the International Federation of clinical chemistry (IFCC) Standard, Lot # 293, Has been demonstrated to recover 100% of this standard.

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

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