

Total Human Apolipoprotein A1 (Apo A1) ELISA Assay

Intended Use:

To quantitate total human Apolipoprotein A1 (Apo A1).

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°C with the exception of the standard, which should be stored frozen.

Patient and Standard Dilutions:

*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 1:10,000 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.

Materials Supplied:

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

TMB/Peroxide substrate color developer III – 12 mL

Sulfuric acid termination reagent (0.5 N) – 12 mL

15X wash buffer concentrate – 2x60 mL

Limitations of the Procedure:

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

Dynamic Range:

0.52 µg/dL – 33.3 µg/dL

Reproducibility:

C.V. 6%-10% depending on region of the standard curve.

Assay Procedure:

*Allow each reagent 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 0.5N sulfuric acid.
9. Zero the microwell reader at 450 nm using the blank 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 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.**