

# TurbiMAX Apolipoprotein A1

## Turbidimetric Immuno Assay (TIA)

### ORDERING INFORMATION

Ref. No.	Pack Size	Presentation
AVAPOA1-25	25 ml	1 x 20 ml/ 1 x 5ml

### INTENDED USE:

TurbiMAX Apolipoprotein A1 is an in-vitro diagnostic kit s use for the Quantitative determination of Apolipoprotein A1 in human Serum and Plasma. This kit is a automated.

### INTENDED USER:

Laboratory Technician

### PRODUCT FEATURES

1. Quantitative Turbidimetric Immuno Assay (TIA)
2. Two liquid reagents (Diluent and Antibody).
3. Lyophilized Calibrator Provided
4. 2 Minutes Fixed Time Assay
5. Linearity : 250 mg/dL

### CLINICAL SIGNIFICANCE:

Apo A-I is the major structural apolipoprotein in HDL and constitutes about 70% of the total protein. Apo A-I is a cofactor for lecithin-cholesterol-acyl- transferase (LCAT), the enzyme responsible for forming cholesteryl esters in plasma and plays an important role in the transport of cholesterol from peripheral tissues to the liver, to be finally excreted. Measurements of Apo A-I concentration is specially important in detecting coronary heart disease risk (CHD) as well as in the diagnostic of hyperlipoproteinemia. Concentrations < 120 mg/L are associated to an increased CHD risk, while concentrations  $\geq$  160 mg/L may even protect from the same risk. Patients with deficiencies in Apo A-I synthesis may highly increase the CHD risk.

Tanger disease, a consequence of an Apo A-I catabolism defect, is characterized by several reduced plasma HDL cholesterol (HDL-c) concentration, abnormal HDL composition and accumulation of cholesteryl esters in many body tissues. Plasma HDL-c and Apo A-I concentrations in homozygotes are very low, while Apo A-II concentration is less than 10% of its normal concentration. Heterozygotes are characterized by half-normal concentration of HDL-c, Apo AI and Apo -II. Current evidence suggests that these patients have increased incidence of CHD.

### PRINCIPLE:

Turbidimetric test for the measurement of apolipoprotein A-I in human serum or plasma. Anti- Apo A-I antibodies when mixed with samples containing Apo A-I , form insoluble complexes. These complexes cause an absorbance change, dependent upon the Apo A-I concentration of the patient sample, that can be quantified by comparison from a calibrator of known Apo A-I concentration

### STORAGE AND STABILITY

All the reagents are stable up to the expiry date mentioned on the labels when the proper storage conditions are maintained.

### KIT COMPONENTS

1. APOA1 Reagent R1
2. APOA1 Reagent R2
3. APOA1 Calibrators (5) : Concentration as stated on the label

### COMPOSITION

Diluent (R1): Tris buffer 20 mmol/L, PEG, pH 8.3. Sodium azide 0.95 g/L.

Reagent (R2) : Goat Serum, Anti Human APO A1, Tris buffer 50 mmol/L. Sodium azide 0.95 g/L.

### REAGENT RECONSTITUTION & STABILITY

Reagent are liquid stable no need for reconstitution.

When the reagent is stored properly at 2-8°C & the contamination avoided, it is stable up to the expiry date mention on the label & kit box.

**APO A1 Calibrator:** Calibrator is available as Lyophilized Calibrator.. **Reconstitute Calibrator with 1.0 ml of Distilled Water and keep it for 30 Minutes.** Mix gently and make a uniform suspension. Reconstituted Calibrator is stable for 60 Days once stored properly at 2-8°C. Aliquot it in to small volumes and

store at 2-8°C for the contamination free use and for good reconstitution stability. Calibrator is stable for 6 Months when frozen at -20°C if the repeated freeze and thaw cycles are avoided. Calibrator needs to be serially diluted as per the procedure mentioned in the Calibrator insert.

### MATERIAL REQUIRED BUT NOT PROVIDED

Laboratory Instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi auto, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/rack, heating bath controls, saline.

### REAGENT DETERIORATION

Discard any turbid reagent if blank reagent absorbance exceeds 1.4 at 340 nm against distilled water.

### WARNING & PRECAUTIONS

- Reagent may contain some non reactive and preservative components. It is recommended to handle carefully, avoiding contact with skin and ingestion.
- Specimen should be considered infectious and handled appropriately.
- Contamination by soap or glycerol will affect this assay.
- Perform the test according to the general " Good Laboratory Practice" GLP

### SPECIMEN COLLECTION & STORAGE

Fresh serum or Plasma. EDTA or Heparin should be used as anti coagulant. Stable 15 days at 2-8°C or 3 months at -20°C.

Samples with presence of fibrin should be centrifuged before testing.

Do not use highly hemolized or lipemic samples.

### SYSTEM PARAMETERS:

Calibration Method	Multi Point -Linear- Spline
Reaction Type (Mode)	Fixed Time /Two Point
Reaction Direction	Increasing
Wave Length	340 nm
Flow Cell Temp.	37°C
Delay Time	20 Seconds
Measuring Time	120 Seconds
Blank	Distilled Water Blank
Reagent Volume	400 $\mu$ l (R1) + 100 $\mu$ l (R2)
Sample Volume)	5 $\mu$ l
Calibrator Concentrations	(On the Vials Lot Specific)
Units	mg/dL
Low normal	110
High normal	170
Linearity	250

### TEST PROCEDURE

Reagent	Calibrator	Sample/Control
Reagent R1	400 $\mu$ l	400 $\mu$ l
Calibrator(1,2,3,4,5)	5 $\mu$ l	----
Sample	—	5 $\mu$ l
Reagent R2	100 $\mu$ l	100 $\mu$ l

- 1) Read absorbance A1 after 20 Seconds. (Delay)
- 2) Incubate and Read the absorbance A2 after 120 Seconds (Measuring)
- 3) Calculate the absorbance differences  $\Delta A = A2 - A1$  for each point of the calibration curve, controls and all unknown samples.
- 4) The concentration of Apo-A1 in the unknown sample can be calculated from  $\Delta A = A2 - A1$
- 5) Using a 3rd order polynomial mathematical model where abscissa (X) is

the  $\Delta A = A_2 - A_1$  and ordinate (Y) is the concentration of Apo-A1 or plotting the values of  $\Delta A = A_2 - A_1$  obtained for every concentration level of the calibrator against the Apo-A1 concentration and interpolating the individual  $\Delta A = A_2 - A_1$  of every sample in the calibration curve.

Calculations with Calibrators/ Calibration Curve/ Result Interpretation:

#### CALCULATION:

The concentration of Apo-A1 in unknown samples is derived from a calibration curve using an appropriate mathematical models such as Multi Point / Linear/Spline. The calibration curve is obtained with 5 calibrators at different levels. Stability of calibration: 4 weeks

#### EXPECTED VALUE

Women 120 – 190 mg/dL  
Men 110 – 170 mg/dL

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

#### CLINICAL INTERPRETATION

Risk of CHD:

Several studies indicate that the Apo-B / Apo-A1 Ratio perfectly reflects the CHD

Men: Lower Risk: <0.7  
Average Risk: 0.7-0.9  
Higher Risk: >0.9

Women: Lower Risk: <0.6  
Average Risk: 0.6-0.8  
Higher Risk: >0.8

Apo-A alone and APO-B alone can not predict the CHD properly. Together when Apo-A1 and Apo- B are estimated as a ratio they are the better risk indicators of CHD. In order to estimate APO-B/APO-A1 Ratio one has to estimate APO A1 and Apo-B too. Avecon offers both APO-A1 and Apo-B Test kits

#### QUALITY CONTROL & CALIBRATION:

Control Sera are recommended to monitor the performance of manual and automated assay procedures.

#### PERFORMANCE CHARACTERISTICS:

##### 1. Linearity

Linearity : 250 mg/dl

##### 2. Sensitivity/ Limit of Detection (LOD)

The lower limit of detection is 0.31 mg/dl

##### 3. Interferences:

Hemoglobin (20 g/L), bilirubin (40 mg/dL), lipemia (< 5 g/L), and rheumatoid factor (800 IU/mL) do not interfere. Other substances may interfere.

**4. Precision:** The reagent has been tested for 20 days, using three levels of serum in a EP5-based study (NCCLS).

#### Intra-Assay

N=20	Mean mg/dl	SD mg/dl	CV%
Control serum 1	27.22	1.1	4.04
Control serum 2	65.74	0.8	1.21
Control serum 3	131.07	1.3	0.99

#### Inter-Assay

N=20	Mean mg/dl	SD mg/dl	CV%
Control serum 1	26.22	1.3	4.95
Control serum 2	66.74	0.9	1.34
Control serum 3	132.07	1.4	1.06

#### 5. Method Comparison:

Results obtained using this reagent (y) were compared to those obtained with a Bayer immunoturbidimetric method. 39 samples ranging from 50 to 200 mg/dL of Apo A-I were assayed. The correlation coefficient (r) was 0.92 and the regression equation  $y = 1.18x - 37.8$ .

#### LIMITATIONS

Measuring range: 0.31-250 mg/dl. Determine samples having higher concentrations manually dilute with 0.9% NaCl or distilled/deionized water (e.g. 1 + 1). Multiply the result by the appropriate dilution factor (e.g. 2).

#### WASTE DISPOSAL

Reagents must be disposed off in accordance with local regulations.














#### NOTES:

Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

#### REFERENCE

1. Clinical Guide to Laboratory Tests, Edited by NW Tietz W B Saunders Co., Philadelphia, 483, 1983.
2. Mahley RW et al. J Lipids Res 1984; 25: 1277-1294.
3. Rifai N Arch Pathol Lab Med 1986; 110: 694-701.
4. Freedman DS et al. N Eng J Med 1986; 315: 721-726.
5. Sakurabayashi I et al. Clinica Chimica Acta 2001; 312: 87-95.
6. Young DS. Effects of disease on clinical laboratory tests, 3th ed. AACC Pres, 1997.
7. Friedman and Young. Effects of disease on clinical laboratory tests, 3th ed. AACC Pres, 1997.

#### Symbols Used on Pack

 REF	Catalogue Number		Warning/Caution
 LOT	Batch No.		In vitro diagnostic device
	Manufacturing Date		Storage Limit
	Expiry Date		Consult instruction for use
	Manufacturer		Keep away from sunlight
	Keep Dry		Do not use if package is damaged
	Contains sufficient no. of test		

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