TurbiMAX Apolipoprotein B

Turbidimetric Immuno Assay (TIA)

ORDERING INFORMATION

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

INTENDED USE

TurbiMAX Apolipoprotein B is an in-vitro diagnostic kit for the quantitative determination of APO lipoprotein B in human Serum and Plasma.

PRODUCT FEATURES

1. Quantitative Turbidimetric Immuno Assay (TIA)
2. Two liquid reagents (Diluent and Reagent R2).
3. Lyophilized Calibrator Provided
4. 2 Minutes Fixed Time Assay
5. Linearity : 250 mg/dL

CLINICAL SIGNIFICANCE:

APO B is the major structural apolipoprotein in VLDL (Very Low Density Lipids), LDL (Low Density Lipids) lipoproteins and chylomicron. The most important function is the transport of rich tryglicerides lipoproteins from liver and intestine to other tissues. Apo B exists in two forms: APO B-100 and APO B-48. APO B-100, the most important component of LDL, is synthesized in the liver and excreted in plasma as part of VLDL. APO B-48, the most important component of chylomicrons, is synthesized in the intestine.

Several studies demonstrated that in people with coronary heart disease (CHD), changes in the serum concentrations of APO A-I and APO B are similar to those for HDL and LDL, respectively and whereas, are somewhat better discriminators of people with CHD than the LDL and HDL cholesterol concentrations.

The hiperbetalipoproteinemia is characterized by increased LDL APO B-100 concentrations with normal or moderately increased concentrations of LDL cholesterol. The ratio of LDL cholesterol to APO B-100 is therefore reduced in these patients.

Defects in the APO B structure or lipoproteins containing APO B prevent the secretion of triglycerides rich intestinal and hepatic lipoproteins; this disorder occurs in abetaliporpteinemia or homozygous hypobetalipoproteinemia.

PRINCIPLE:

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

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the labels when stored at 2-8ºC and the contaminations is prevented during their use. Do not freeze the latex and diluent.

KIT COMPONENTS

1. Diluent Reagent R1
2. Reagent R2
3. APO B Calibrators : 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 B, 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 B 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.

Calibrator Traceability

The Assay and the values of the Calibrator Concentration have been standardized against the certified reference Materials WHO/IFCC SP1-01 (CDC,USA)

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 1.2 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 2-8ºC or 3 months at –20ºC.
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 µl (R1) + 100 µl (R2)
Sample Volume)	10 µl
Calibrator Concentrations	(On the Vials Lot Specific)
Units	mg/dL
Low normal	80
High normal	155
Linearity	250

TEST PROCEDURE

Reagent	Calibrator	Sample/Control
Reagent R1	400 µl	400 µl
Calibrator(1,2,3,4,5)	10 µl	----
Sample	—	10 µl
Reagent R2	100 µl	100 µl

1) Read absorbance A1 after 20 Seconds. (Delay)
2) Incubate and Read the absorbance A2 after 120 Seconds (Measuring)
3) Calculate the absorbance differences ΔA=A2–A1 for each point of the calibration curve, controls and all unknown samples.

4) The concentration of Apo-B in the unknown sample can be calculated from ΔA=A2–A1

5) Using a 3rd order polynomial mathematical model where abscissa (X) is the ΔA=A2–A1 and ordinate (Y) is the concentration of Apo-B or plotting the values of ΔA=A2–A1 obtained for every concentration level of the calibrator against the Apo-B concentration and interpolating the individual ΔA=A2–A1 of every sample in the calibration curve.

Calculations with Calibrators/ Calibration Curve/ Result Interpretation:

CALCULATION:
The concentration of Apo-B 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 - APO-B
Mean values"
Men : Desirable < 150 mg/dL
Women Desirable < 155 mg/dL

Each laboratory should check if the reference ranges aretransferable to its own patient population and determine ownreference 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.
Each laboratory should establish its own Quality Control scheme.

PERFORMANCE CHARACTERESTICS:

1. Linearity
Linearity : 250 mg/dL

2. Sensitivity/ Limit of Detection (LOD)
The lower limit of detection is 3.02 mg/dL

3.Interferences:
Hemoglobin (20 g/L), bilirrubin (40 mg/dL), lipemia (2.5 g/L), and rheumatoid factor (800 UI/mL) do not interfere. Other substances may interfere

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

Intra-Assay

N=10	Mean (mg/dl)	SD (mg/dl)	CV%
Control serum 1	23.92	0.96	4.01
Control serum 2	119.07	1.42	1.19

Inter-Assay

N=10	Mean (mg/dl)	SD (mg/dl)	CV%
Control serum 1	24.2	1.2	4.95
Control serum 2	119.7	1.52	1.27

5. Method Comparison:
Results obtained using this reagent (y) were compared to those obtained with a similar immunoturbidimetric method. 48 samples ranging from 25 to 190 mg/dL of APO B were assayed. The correlation coefficient (r) was 0.982 and the regression equation y = 0.996x + 5.112

LIMITATIONS
3.02 - 250 mg/dL, under the described assay conditions. Samples with higher concentrations, should be diluted 1/5 in NaCl 9 g/L and retested again. The linearity limit depends on the sample / reagent ratio. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.

WASTE DISPOSAL

Reagents must be disposed off in accordance with local regulations.

REFERENCE

1. Clinical Guide to Laboratory Tests, Edited by NW Tietz W B Saunders Co ., Phipladelphia, 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, 3tn ed .AACC Pres, 1997.

Symbols Used on Pack

REF	Catalogue Number		Warning/Caution
LOT	Batch No.	IVD	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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