

## Intended Use

For *in vitro* diagnostic use only. This reagent is intended for use in the quantitative determination of apolipoprotein B (apo B) in human serum by immunoturbidimetric analysis to aid in the assessment of individuals who are at risk for developing coronary artery disease.

## Clinical Significance

Human plasma low density lipoproteins (LDL) are particles that contain by weight percent approximately 25% protein, 40% cholesterol, 25% phospholipid, and 10% triglyceride.<sup>1</sup> Apolipoprotein B-100 (apo B) is the major protein of LDL comprising over 90% of the particle's protein mass. Increased levels of plasma LDL cholesterol have been associated with an enhanced risk for premature coronary artery disease (CAD).<sup>2</sup>

There is general agreement that apo B plays an essential role in lipid transport and metabolism through its interactions with specific cellular membrane receptors and by its inhibition of the rate controlling enzyme in cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl coenzyme A reductase.<sup>3</sup> Numerous studies have indicated that measurements of plasma apo B are useful in assessing CAD risk<sup>4,5</sup> and some studies have suggested that apo B measurements in combination with apo A1 measurements may be better predictors for premature CAD than other lipid parameters.<sup>6,7</sup> Also, quantitation of apo B is important in identifying those individuals who have elevated apo B levels (hyperapobetalipoproteinemia) despite having normal plasma levels of LDL cholesterol.<sup>7</sup> Patients with angiographically documented CAD were found to have elevated apo B levels and decreased apo A1 levels compared with individuals without the disease.<sup>10</sup>

## Principle

Immunoturbidimetric methods for apo B quantitation have been described.<sup>8,9</sup> An insoluble turbid immunoprecipitate is formed by the reaction between the apo B antigen in human serum and the specific antibody contained in the R2 reagent. The resulting turbidity is measured spectrophotometrically at 340 nm and the apo B in the serum is determined from a calibration curve obtained by using the four level calibrator set used to calibrate the chemistry analyzer (See Procedure).

The R2 Reagent of this method was prepared in house from antiserum produced in goats against purified human apo B derived from the LDL fraction (d=1.030-1.050) of pooled human serum. The antiserum was found to be monospecific when tested by immunoelectrophoresis against whole human serum.

The R1 Reagent is a buffered solution containing polyethylene glycol, stabilizers and preservatives.

The liquid calibrators were prepared from pooled human serum and contain apo B levels sufficient for quantitative and quality control of normal and abnormal samples. The apo B protein in these sera has been assigned concentration values with the use of the International Federation of Clinical Chemistry (IFCC) proposed Standard Reference Material, SP3-07, and by participation in the IFCC/CDC directed Standardization Program.

## Reagents

1. R1 Reagent: Tris (pH 7.0) 100mmol/L, Polyethylene glycol (PEG), detergents, stabilizers.
2. R2 Reagent: Tris (pH 7.0) 100mmol/L, anti-human apolipoprotein B antibody (goat) with stabilizers.

NOTE: See Precautions section for information regarding handling of kit components.

## Reagent Preparation

Reagents are supplied in ready-to-use liquid form. Allow both reagents to equilibrate to room temperature prior to use.

## Reagent Storage and Stability

1. The apo B reagents are stable until the expiration date shown on the labels. Store the kit in a refrigerator at 2-8°C. Do Not Freeze.
2. Keep reagent vials tightly capped to avoid microbial contamination and evaporation.

## Precautions

1. Avoid ingestion of reagent and contact with skin. The toxicity of these reagents has not been established.
2. The reagent contains 0.1% sodium azide which can react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water.<sup>11</sup>
3. Reagents and calibrators intended for use with this reagent kit must not be stored frozen and should not be allowed to stand for repeatedly long periods of time (over 8 hours) at room temperature. Keep reagents and calibrator vials tightly capped at all times when not in use to avoid microbial contamination and evaporation.

## Specimen Collection

Serum, heparinized plasma or EDTA plasma

Stability : 5 days at 15-25°C  
14 days at 2-8°C  
90 days at -20°C

Avoid repeated freezing and thawing as this may result in lipoprotein denaturation. Discard contaminated specimens.

## Materials Required but not Provided

1. Suitable chemistry analyzer. See Procedure and the specific instrument Application Guide for required reagent volumes and instrument parameters. Contact the manufacturer's Technical Service Department for available application guides.
2. **Apo A1/B Serum Calibrators.** This four level human serum calibrator set must be used with the reagents of this kit. **The manufacturer does not recommend the use of calibrators from other sources with the reagents of this kit.**
3. Controls with known apolipoprotein values.
4. 0.9% NaCl solution

## Materials Provided

1. Apo B Reagent 1 (1 x 120ml)
2. Apo B Reagent 2 (1 x 30ml)

## Procedure

TEST NAME	[APOB]
ASSAY CODE	[2-POINT]:[23]-[50]
SAMPLE VOLUME	[3] [2]
R1 VOLUME	[300] [**] [NO]
R2 VOLUME	[75] [**] [No]
WAVELENGTH	[700] [340]
CALIBRATION	[NONLINEAR] [1] [5]
STD (1) CONC-POS	[0] [1]
STD (2) CONC-POS	[*] [*]

# Apolipoprotein B Reagent Set

STD (3) CONC-POS	[*] [*]
STD (4) CONC-POS	[*] [*]
STD (5) CONC-POS	[*] [*]
STD (6) CONC-POS	[*] [*]
SD LIMIT	[500]
DUPLICATE LIMIT	[500]
SENSITIVITY LIMIT	[0]
ABS LIMIT (INC/DEC)	[32000] [INCREASE]
PROZONE LIMIT	[0] [LOWER]
EXPECTED VALUE	[*] [*]
PANIC VALUE	[*] [*]
INSTRUMENT FACTOR	[1.0]

1. Allow reagents, calibrator sera, control sera, and samples to equilibrate to room temperature prior to use.
2. Calibrate the clinical chemistry analyzer in accordance with the instrument user's manual and the above parameters.

## Interferences / Limitations

1. Apolipoprotein B is a specific assay for Apo B through its use of specific antibodies to Apo B. No interference was observed with ascorbic acid levels up to 20 mg/dl, bilirubin up to 17.5 mg/dl, hemoglobin up to 500 mg/dl and triglycerides up to 900 mg/dl.
2. No cross reaction with apolipoprotein A1 or apolipoprotein A2 was observed under test conditions.

## Quality Control

The reliability of test results must be monitored by routine use of control sera containing assayed levels of apo B. These QC materials should be assayed in every run and treated as patient samples. For interpretation of results refer to target values and limits of performance provided with the controls. If QC results are not expected: 1) Review relevant applications guide to ensure that the test was performed in accordance with the prescribed procedure, 2) Check to see that the materials used have not expired, 3) If necessary, rerun the controls. For further assistance contact the manufacturer.

## Results

The concentration of apo B for unknown serum samples is obtained by non-linear data reduction which is automatically calculated by the clinical chemistry analyzer. Results are then printed by the analyzer in appropriate units (mg/dL or g/L). For instrument specific calculations, see the appropriate Operator's Manual concerning information on the required non-linear math model for calibration curve fit.

## Assay Range

The test has been developed to determine the concentrations of apolipoprotein B within a measuring range from 3 – 250 mg/dl. When values exceed this range, samples should be diluted 1:1 with 0.9% saline solution and the result multiplied by 2.

**The assay range for this reagent is 3-250 mg/dL**

## Prozone Limit

No prozone effect was observed up to an apolipoprotein B value of 800 mg/dl.

## Sensitivity/Limit of Detection

The lower limit of detection is 3 mg/dl.

## Expected Values

The reported reference ranges for apo B are: 60-150 mg/dL for females and 63-152 mg/dL for males.<sup>12</sup> It is recommended that each laboratory establish its own "normal" range.

## Performance Characteristics

The following categories list typical data obtained when using the Hitachi 717 instrument system. The actual results obtained may vary when other automated instrument procedures are employed.

## Comparisons

A comparison between this apolipoprotein B (y) and a commercially available test (x) using 112 samples gave the following results:  $y = 0.999x + 9.85$ .  $r = 0.956$

## Precision

Two of the serum samples used in the method comparison study above were analyzed for precision of apo B. Apo B, mg/dl:

Within Run				Run to Run			
Mean	S.D.	C.V.%	N	Mean	S.D.	C.V.%	N
34.1	0.6	1.8	20	199.0	3.4	1.8	21
176.7	1.9	1.1	20	35.8	0.7	1.9	21

## References

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