LIPOPROTEIN (a) (Lp (a))

RX SERIES

INTENDED USE

Immunoturbidimetric assay for the quantitative *in vitro* determination of Lipoprotein (a) in human serum or plasma.
This product is suitable for use on RX **series** instruments which includes the RX **daytona** and RX i**mola**.

Cat. No.		
LP 3403	R1. Buffer	1 x 10 ml
	R2. Latex Reagent	1 x 6 ml

GTIN: 05055273204421

CLINICAL SIGNIFICANCE[1]

Lipoprotein (a) determination is intended for use in conjunction with clinical evaluation, patient risk assessment and other lipid tests to evaluate disorders of lipid metabolism and to assess coronary heart disease in specific populations.

PRINCIPLE

Agglutination occurs due to an antigen-antibody reaction between Lp(a) in a sample and anti-Lp(a) antibody adsorbed to latex particles. This agglutination is detected as an absorbance change at 700 nm proportional to the concentration of Lp(a) in the sample.

Note: This product is licensed from Denka Seiken.

SAMPLE COLLECTION AND PREPARATION[2]

Collect serum using standard sampling tubes and plasma using tubes containing Li heparin, Na heparin, Na EDTA, K EDTA, citrate.

SAMPLE STORAGE AND STABILITY[3]

Samples may be stored at 4°C for 14 days without significant decrease. For long term storage the samples should be stored at -20°C or -70°C.

REAGENT COMPOSITION

Contents	Initial Concentration
R1. Buffer	
Glycine	0.17M
Sodium Chloride	1.08M
Sodium ethylenediamine tetra acetic acid disodium salt dihydrate	0.05M
Sodium azide	≤0.09% w/v
R2. Latex Reagent	
Glycine	0.17M
Sodium Chloride	0.1M
Suspension of latex particles coated with anti-lp(a) antibodies	0.5%
Sodium azide	≤0.09% w/v

SAFETY PRECAUTIONS AND WARNINGS

For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Safety Data Sheets are available on request.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS

R1. Buffer
Buffer is ready for use and is stable up to the expiry date when stored at +2°C to +8°C protected from light.

R2. Latex Reagent
Latex Reagent is ready for use and stable up to the expiry date when stored at +2°C to +8°C protected from light. Invert several times before use, avoiding the formation of foam.

Reagent 1 = Buffer
Reagent 2 = Latex Reagent

MATERIALS PROVIDED

Buffer
Latex Reagent

MATERIALS REQUIRED BUT NOT PROVIDED

Randox Lipoprotein (a) Calibrator Series Cat. No. LP 3404
Randox Lipoprotein (a) Control Level 3 Cat. No. LP 3406
Randox Lipid Controls:-

Level 1	LE 2661 or LE 2668
Level 2	LE 2662 or LE 2669
Level 3	LE 2663 or LE 2670

NB all 3 levels of lipid control contain Lp(a) with concentrations in the normal range.

PROCEDURE NOTES

The Chemistry parameters for Randox Dedicated RX **series** Assays are predefined on the hard drive of the analyser PC. The required programs should be downloaded to the analyser software. Please note that the predefined chemistry parameters use SI units. If alternative units are required, these can be edited by the user. In this case, the technical range should be edited in accordance with the users selected units. All necessary instructions are encoded on the bar code. If the barcode cannot be read by the analyser, enter manually the series of numbers given beneath the barcode. If problems continue contact Randox Laboratories Technical Services, Northern Ireland +44 (0) 28 9445 1070

CALIBRATION

The use of Randox Lp(a) Calibrator Series is recommended for calibration. Saline is used as S1 and Cal 1-5 as S2-S6. A multi-point calibration is recommended, with change of reagent lot/bottle or as indicated by quality control procedures.

Instrument Specific nmol/l values are available for various analysers.

QUALITY CONTROL

Randox Lipoprotein(a) Control Level 3 and a Randox Lipid control are recommended for quality control to monitor accuracy and precision. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water. Contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Technical Services, Northern Ireland + 44 (0) 28 9445 1070.

Quality control requirements should be determined in conformance with government regulations or accreditation requirements.

SPECIFICITY/INTERFERENCE

The following analytes were tested up to the noted levels and did not cause interferences:

Intralipid®	5%
Bilirubin	35 mg/dl
Haemoglobin	1040 mg/dl
Ascorbic Acid	50 mg/dl
Triglycerides	493mg/dl
Plasminogen	200 mg/dl
Apolipoprotein B	200 mg/dl

	50nmol/l	95 nmol/l
Intralipid® (mg/dl)	2000	2000
Bilirubin Conjugated (mg/dl)	60	60
Bilirubin Unconjugated (mg/dl)	60	60
Haemoglobin (mg/dl)	1000	1000
Triglycerides (mg/dl)	2000	2000
RF	400	400

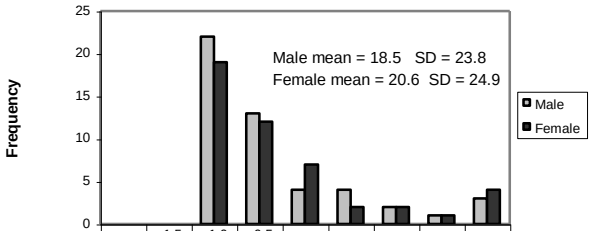
Physiological changes in serum or plasma analyte concentrations can be caused by a number of substances. Comprehensive discussion of possible interfering substances, their serum or plasma concentrations, and their possible physiological involvements is beyond the scope of this document. The listed reference contains specific details on known potential interfering substances[4]. The user must remain vigilant to the possible effect on results of unknown interferences from medications or endogenous substances. All patient results must be evaluated in light of the total clinical status of the patient.

NORMAL RANGE [1,5,6,7]

ADULTS < 30 mg/dl or < /= 75 nmol/l

The above reference range was established based on a sample of 96 Caucasian individuals comprising 49 males (age range 17-90 years; mean = 55 years) and 47 females (age range 13-84 years; mean = 55 years) resident in Northern Ireland. The population tested was an ambulatory population with no history of coronary disease. Results showed a mean Lp(a) value of 18.5 mg/dl for males and 20.6 mg/dl for females. Reference ranges have not been established for this assay for different ethnic populations or disease states.

Lipoprotein (a) In 96 N. Ireland Individuals



	-2 to -1.5 SD	-1.5 to -1.0 SD	-1.0 to -0.5 SD	-0.5 to 0 SD	0 to 0.5 SD	0.5 to 1.0 SD	1.0 to 1.5 SD	1.5 to 2.0 SD	> 2 SD
Male	0	0	22	13	4	4	2	1	3
Female	0	0	19	12	7	2	2	1	4

SD from Mean

Lp(a) concentrations have been shown to be genetically determined and to vary with ethnic populations. One study carried out in the United States showed that mean plasma levels of Lp(a) were approximately twice as high in African people or people of African descent compared to levels in Caucasians[4]. Also, the distribution of Lp(a) is less skewed in African people or people of African descent than in Caucasians[4]. Other studies have also shown no difference in Lp(a) levels between men (mean = 14mg/dl) and women (mean =15mg/dl)[5]. Levels of Lp(a) have been shown not to differ significantly between pre- and post-menopausal Caucasian women[5].

It is therefore recommended that each laboratory establish its own reference range to reflect the age, race, sex, diet and geographical location of the population.

SPECIFIC PERFORMANCE CHARACTERISTICS

The mg/dl performance data was obtained using a RX **daytona** analyser at 37°C. The nmol/l performance data was obtained using a RX **imola** analyser at 37°C.

ASSAY RANGE

The range of this assay is approximately 3 - 90 mg/dl (5.23 - 206 nmol/l). In the event of a rerun the upper limit is extended to approximately 180 mg/dl.

Alternatively if the sample concentration exceeds the assay range, dilute the sample 1+2 with 0.9% NaCl solution and reassay. Multiply the result by 3.

These values are dependent on the lot specific values of the calibrators in use.

PROZONE EFFECTS

Antigen excess effects are not noted up to 341 mg/dl (694 nmol/l).

SENSITIVITY (mg/dl)
The minimum detectable concentration with an acceptable % coefficient of variation was determined as 3.4 mg/dl.
The minimum detectable concentration with an acceptable coefficient of variation (in percentage) has been determined to be 3,4 mg/dl.

SENSITIVITY (nmol/l)
The limit of Quantitation (LoQ), the limit of Detection (LoD) and the limit of Blank (LoB) were determined consistent with CLSI guidelines EP17-A. LoQ is the lowest concentration that can be detected with ≤40% Total error. LoD is the lowest concentration that can be detected to determine the presence or absence of LP(a). LoB is the highest concentration that is likely to be observed in a blank sample.

	nmol/l
Limit of Blank	0.61
Limit of Detection	1.17
Limit of Quantitation	5.23

PRECISION (mg/dl)

Intra Assay precision

	Level 1	Level 2	Level 3
Mean (mg/dl)	19.9	27.4	59.1
SD	0.46	0.69	1.02
CV(%)	2.3	2.54	1.72
n	20	20	20

Inter Assay precision

	Level 1	Level 2	Level 3
Mean (mg/dl)	22.8	27.5	57.7
SD	1.39	1.17	1.73
CV(%)	6.09	4.14	2.99
n	20	20	20

PRECISION (nmol/l)

Within run precision

	Level 1	Level 2	Level 3
Mean (nmol/l)	24.6	59.27	91.41
SD	0.52	0.72	0.97
CV(%)	2.1	1.2	1.1
n	200	200	200

Total precision

	Level 1	Level 2	Level 3
Mean (nmol/l)	24.6	59.27	91.41
SD	0.74	1.88	2.81
CV(%)	3	3.2	3.1
n	200	200	200

CORRELATION (mg/dl)
This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:

Y = 0.97 X + 2.17
and a correlation coefficient of r = 0.995

29 patient samples were analyzed spanning the range 3.49 to 81.29 mg/dl.

The Randox Lp (a) test kit shows minimum apo (a) size related bias. Size heterogeneity of apo (a) can affect to varying degrees the outcome of other commercially available kits.[6]

CORRELATION (nmol/l)
Y = 1.06 X + 0.87
and a correlation coefficient of r = 0.998

syx = 3.21

102 patient samples were analyzed spanning the range 4.84 to 203.48.

SERUM/PLASMA COMPARISON
The Randox method was used to compare serum samples (X) to plasma samples (Y) collected into tubes containing Li heparin, Na heparin, Na EDTA, K EDTA or citrate. 56 samples were tested. The data was subjected to linear regression analysis.

Results:

(i) Serum/Plasma (Li heparin):

Sample range:	2 – 77.3 mg/dl
Linear regression analysis:	y = 0.956x – 1.199
Correlation coefficient r:	0.996

(ii) Serum/Plasma (Na heparin)

Sample range:	2.3 – 78.3 mg/dl
Linear regression analysis:	y = 0.958x – 0.522
Correlation coefficient:	0.996

(iii) Serum/Plasma (Na EDTA)

Sample range:	1.7 – 78.8 mg/dl
Linear regression analysis:	y = 0.972x + 0.023
Correlation coefficient:	0.999

(iv) Serum/Plasma (K EDTA)

Sample range:	1.8 – 79.5 mg/dl
Linear regression analysis:	y = 0.981x + 0.085
Correlation coefficient:	0.999

(v) Serum/Plasma (Citrate)

Sample range:	2.0 – 79.4 mg/dl
Linear regression analysis:	y = 0.963x + 0.065
Correlation coefficient:	0.999

LIMITATIONS

1. Performance of this assay was not tested with age - matched pairs in a diseased population.
2. Normal range values for this assay have not been established for African - American populations.
3. This assay has not been tested for interference by Statin Therapy.
4. Intake of alcohol, aspirin, niacin and estrogen supplements have the potential of causing a misrepresentation of the true LP(a) concentrations.

REFERENCES

1. Lothar Thomas. Clinical Laboratory Diagnostics. 1st Edition. TH Books.
2. Tietz NW Clinical Guide to Laboratory Tests. Philadelphia, Pa: WB Sanders, 1995: 442 - 444.
3. Kronenberg, Lobentanz, Konig, Utermann and Dieplinger; (1994), *Journal of Lipid Research* **35**:1318-1328.
4. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Washington, DC: AACC Press; 2000.
5. Marcovina, S.M., Albers, J.J., Wijsman, E., Zhang, Z., Chapman, N.H. and Kennedy, H. (1996). Differences in Lp(a) concentrations and apo(a) polymorphs between black and white Americans. *Journal of Lipid Research* **37**: 2569 – 2585
6. Jenner, J.L., Ordovas, J.m., Lamon-Fava, S. *et al*. (1993). Effects of Age, Sex and Menopausal Status on Plasma Lipoprotein (a) Levels. The Framingham Offspring Study. Circulation **87**: 1135 – 1141.
7. Santica M Marcovina, Marlys L Koschinsky, John J Albers, Sonia Skarlatos, Report of the National Heart, Lung and Blood Institute Workshop on Lipoprotein(a) and Cardiovascular Disease: Recent Advances and Future Directions, *Clinical Chemistry*, Volume 49, Issue II, I November 2003, Pages 1785 – 1796.
8. Marcovina, S.M., Albers, J.J., Scanu, A.M. *et al*. (2000) Use of a Reference Material Proposed by the International Federation of Clinical Chemistry and Laboratory Medicine to Evaluate Analytical Methods for the Determination of Plasma Lipoprotein (a). *Clinical Chemistry* **46: (12)** 1956 – 1967.

The presence of a vertical bar in the margin indicates a technical update from the previous revision.

EC REP

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