ALT

Alanine Aminotransferase
IFCC
MANUAL
RX MONZA

INTENDED USE

For the quantitative *in vitro* determination of Alanine Aminotransferase (ALT) in serum and plasma. This product is suitable for manual use and on the RX **monza** analyser.

Cat. No.		
AL 1205	R1a. Buffer/Substrate	1 x 105 ml
10 x 10 ml	R1b. Enzyme/Coenzyme/ α-oxoglutarate	10 x 10 ml
GTIN:	05055273200164	
AL 1268	R1a. Buffer/Substrate	1 x 105 ml
5 x 20 ml	R1b. Enzyme/Coenzyme/ α-oxoglutarate	5 x 20 ml
GTIN:	05055273200171	

UV METHOD

This is an optimised standard method according to the concentrations recommended by the IFCC.

PRINCIPLE

$$\alpha\text{-oxoglutarate} + \text{L-alanine} \xrightarrow{\text{ALT}} \text{L-glutamate} + \text{pyruvate}$$

$$\text{pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LD}} \text{L-lactate} + \text{NAD}^+$$

SAMPLE

Serum, heparinized or EDTA plasma.

REAGENT COMPOSITION

Contents		Concentration in the Test
R1a.	**Buffer/Substrate**	
	Tris buffer	100 mmol/l, pH 7.5
	L-alanine	0.6 mol/l
R1b.	**Enzyme/Coenzyme/ α-oxoglutarate**	
	α-oxoglutarate	15 mmol/l
	LD	≥1.2 U/ml
	NADH	0.18 mmol/l

SAFETY PRECAUTIONS AND WARNINGS

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

Solution R1a contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

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

R1a. Buffer/Substrate
Contents ready for use. Stable up to the expiry date when stored at +2 to +8°C.

R1b. Enzyme/Coenzyme/ α-oxoglutarate
Reconstitute one vial of Enzyme/Coenzyme/ α-oxoglutarate R1b with the appropriate volume of Buffer/Substrate R1a:
10 ml for the **10 x 10 ml** kit (AL 1205)
20 ml for the **5 x 20 ml** kit (AL 1268)
Stable for 14 days at +2 to +8°C or 24 hours at +15 to +25°C.

R1 = Buffer/Substrate/Enzyme/Coenzyme/I-oxoglutarate
R2 = None

MATERIALS PROVIDED

Buffer/Substrate
Enzyme/Coenzyme/ α-oxoglutarate

MATERIALS REQUIRED BUT NOT PROVIDED

Randox Assayed Multisera Level 2 (Cat. No. HN 1530) and Level 3 (Cat. No. HE 1532)
Randox Calibration Serum Level 3 (Cat. No. CAL 2351)
RX **series** Saline (Cat. No. SA 3854)

PROCEDURE

Aspirate fresh ddH_2O and perform a new Gain Calibration in flow cell mode. Select ALT in the Run Test screen and carry out a water blank as instructed.

Pipette into a test tube:	
Sample	0.05 ml
Reagent	0.5 ml

Mix and aspirate into the Rx Monza.

CALIBRATION FOR RX MONZA

The use of Saline and Randox Calibration Serum Level 3 is recommended for calibration. Calibration is recommended with change in reagent lot or as indicated by quality control procedures.

FOR MANUAL USE

Wavelength:	340 nm (Hg 334 nm or Hg 365 nm)
Cuvette:	1 cm light path
Temperature:	30/37°C
Measurement:	against air

Pipette into cuvette:

	Macro	Micro
Sample	0.2 ml	0.1 ml
R1. Enzyme/Coenzyme/ α-oxoglutarate	2.0 ml	1.0 ml

Mix, read initial absorbance after 1 min.
Read again after 1, 2, and 3 min. Note if the absorbance change per minute is between
0.11 and 0.16 at 340 nm/Hg 334 nm
0.06 and 0.08 at Hg 365 nm
use only the values for the first 2 minutes for the calculation.

MANUAL CALCULATION

To calculate the ALT activity, use the following formulae:

U/l = 1746 x –A 340 nm/min
U/l = 1780 x –A Hg 334 nm/min
U/l = 3235 x –A Hg 365 nm/min

STANDARDISATION

Randox Calibration Serum Level 3 is traceable to ALT reference material JSCC TS01.

QUALITY CONTROL

Randox Assayed Multisera, Level 2 and Level 3 are recommended for daily quality control. 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.

INTERFERENCE

Avoid haemolysis as it interferes with the assay.

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 [2]. 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 VALUES IN SERUM [1]

	25°C	30°C	37°C
Men	up to 22 U/l	up to 29 U/l	up to 40 U/l
Women	up to 17 U/l	up to 22 U/l	up to 31 U/l

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

SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance data were obtained using an Rx Monza analyser running at 37°C.

SENSITIVITY

The minimum detectable concentration of ALT with an acceptable level of precision was determined as 7.99 U/l.

LINEARITY

This method is linear up to 500 U/l. If the sample concentration exceeds this value, dilute the sample 1+x with 0.9% NaCl solution and reassay. Multiply the result by x+1.

PRECISION

Intra Assay

	Level 2	Level 3
Mean (U/l)	38.9	134
SD	1.79	1.59
CV (%)	4.61	1.19
n	20	20

Inter Assay

	Level 2	Level 3
Mean (U/l)	38.9	134
SD	1.78	5.22
CV (%)	4.58	3.90
n	20	20

CORRELATION

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

Y = 0.99X + 17.2
and a correlation coefficient of r = 0.9935

47 patient samples were analysed spanning the range 18 to 522 U/l.

REFERENCE

1. International Federation of Clinical Chemistry, Scientific Committee. *J. Clin. Chem. Clin. Biochem*. 1980; **18:** 521-534.
2. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Washington, DC: AACC Press; 2000.

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

EC REP Randox Teoranta, Meenmore, Dungloe, Donegal, F94 TV06, Ireland

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