RANDOX

ALDOLASE (ALS)

Fructose-1,6-diphosphate aldolase
MANUAL
RX MONZA

INTENDED USE

For the quantitative *in vitro* determination of Aldolase in serum or plasma. This product is suitable for Manual use and on the Rx Monza analyser.

Cat. No.		
AD 189	R1. Buffer/Substrate	5 x 20 ml
5 x 20 ml	R2. NADH	2 x 1 ml
	R3. GDH/TIM/LDH	1 vial

PRINCIPLE

Aldolase converts fructose-1,6-diphosphate (F-1,6-DP) to glyceraldehyde-3-phosphate (GAP) and dihydroxyacetone phosphate (DAP). The addition of triosephosphate isomerase (TIM), glycerolphosphate dehydrogenase (GDH) and NADH converts the dihydroxyacetone phosphate to glycerol-1-phosphate. The rate of the aldolase reaction is measured by the decrease in absorbance at 340 nm as a consequence of the conversion of NADH to NAD^+.

$$\text{F-1,6-DP} \xrightarrow{\text{Aldolase}} \text{GAP} + \text{DAP}$$

$$\text{GAP} \xrightarrow{\text{TIM}} \text{DAP}$$

$$2\text{DAP} + 2\text{NADH} + 2\text{H}^+ \xrightarrow{\text{GDH}} 2\text{Glycerol-1-phosphate} + 2\text{NAD}^+$$

SAMPLE

Serum, heparinized plasma or EDTA plasma.

REAGENT COMPOSITION

Contents	Concentration in the Test
R1. Buffer/Substrate	
Collidine buffer	51 mmol/l, pH 7.4
Mono-iodoacetate	0.27 mmol/l
F-1,6-DP	2.7 mmol/l
R2. NADH	0.23 mmol/l
R3. GDH/TIM/LDH	
GDH	≥ 326 mU/ml
TIM	≥ 4.35 U/ml
LDH	≥ 616 mU/ml
Ammonium Sulphate	> 35%

SAFETY PRECAUTIONS AND WARNINGS

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

Health and 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/Substrate
Reconstitute one vial of Buffer/Substrate R1 with **20 ml** of redistilled water. Stable for two weeks at +2 to +8°C.

R2. NADH
Reconstitute one vial of NADH R2 with **1 ml** of redistilled water. Stable for 4 weeks at +2 to +8°C.

R3. GDH/TIM/LDH
Contents ready for use. Stable up to the expiry date specified when stored at +2 to +8°C.

STABILITY AND PREPARATION OF WORKING REAGENT FOR RX MONZA

Prepare the working reagent as given in the table below:-

Buffer/Substrate R1	NADH R2	GDH/TIM/LDH R3
2.5ml	0.05ml	0.01ml
5.0ml	0.1ml	0.02ml
10.0ml	0.2ml	0.04ml

Stable for 8 hours at +2 to +8°C.

MATERIALS PROVIDED

Buffer/Substrate
NADH
GDH/TIM/LDH

MATERIALS REQUIRED BUT NOT PROVIDED

Randox Aldolase Calibrator (Cat. No. AD 5000)
Randox Aldolase Control Level 2 (Cat. No. AD 5001) and Level 3 (Cat. No. AD 5002)
0.9% NaCl Solution.

PROCEDURE

Select ALS in the Run Test screen and carry out a water blank as instructed.

Pipette into a test tube:

	SO*	Standard S1	Sample
ddH2O	35µl	---	---
ALS CAL	---	35µl	---
Sample	---	---	35µl
Working Reagent	450µl	450µl	450µl

Mix, incubate for 5 min at +37°C or 10 min at 20-25 °C and aspirate into the Rx Monza.

*reagent blank

CALIBRATION FOR RX MONZA

The use of Saline and Randox Aldolase Calibration Serum 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 365 nm, or Hg 334 nm)
Cuvette:	1 cm light path
Temperature:	37°C
Measurement:	against sample blank

Pipette into test tubes:

	Sample blank	Calibrator	Sample
Sample	0.2 ml	0.2 ml	0.2 ml
Buffer/Substrate (R1)	-	2.50 ml	2.50 ml
0.9% NaCl Solution	2.50 ml	-	-
NADH (R2)	-	0.05 ml	0.05 ml
GDH/TIM/LDH (R3)	-	0.01 ml	0.01 ml

Mix sample, R1, R2 and R3 and incubate for 5 minutes at 37°C. Read absorbance A_1 against sample blank. Allow to stand at 37°C for exactly 20 minutes after first reading and then measure absorbance A_2 against blank.
* If A_1 <0.95, dilute 1+1 with 0.9% NaCl and reassay. Multiply the result by 2.

Randox Laboratories Limited, 55 Diamond Road, Crumlin, County Antrim, BT29 4QY, United Kingdom
T +44 (0) 28 9442 2413 **F** +44 (0) 28 9445 2912 **E** applications@randox.com **I** www.randox.com

MANUAL CALCULATION

To calculate Aldolase activity use the following formula:

$$\frac{(A_1 - A_2)\ \text{Sample}}{(A_1 - A_2)\ \text{Calibrator}} \times \text{Conc. of calibrator}$$

QUALITY CONTROL

Randox Aldolase Control 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 Customer Technical Support, Northern Ireland (028) 94422413.

INTERFERENCE

Haemolysis interferes with the test.

REFERENCE VALUES [1]

Serum: up to 7.6 U/l (37°C)

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 characteristics were obtained using an Rx Monza analyzer in flow cell mode at 37°C.

LINEARITY

The method is linear up to a concentration of 106U/l. If the sample concentration exceeds this value, dilute the sample 1+4 with 0.9% NaCl solution and reassay. Multiply the result by 5.

SENSITIVITY

The minimum detectable concentration of Aldolase with an acceptable level of precision was determined as 1.73U/l.

PRECISION

Within run precision

	Level 2	Level 3
Mean (U/l)	6.36	19.1
SD	0.295	0.854
CV(%)	4.64	4.47
n	20	20

Between run precision

	Level 2	Level 3
Mean (U/l)	6.36	19.1
SD	0.402	1.35
CV(%)	6.32	7.09
n	20	20

CORRELATION

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

$Y = 0.9815\ X + 0.183$
and a correlation coefficient of $r = 0.9917$.

42 Samples were analysed spanning the range 2.46 to 89.86 U/l.

REFERENCE

1. Feissli, S., *et al*., (1966). Klin. Wschr. **44:** 390.

Revised 15 Sep 10 bm
Rev. 001