ALP

Alkaline Phosphatase
MANUAL
RX MONZA

INTENDED USE

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

Cat. No.		
AP 542	R1a. Buffer	1 x 70 ml
20 x 3 ml	R1b. Substrate	20 x 3 ml
GTIN:	05055273200386	
AP 307	R1a. Buffer	1 x 105 ml
10 x 10 ml	R1b. Substrate	10 x 10 ml
GTIN:	05055273200317	

COLORIMETRIC METHOD[1]

This is an optimized standard method according to the recommendations of the Deutsche Gesellschaft für Klinische Chemie.

PRINCIPLE

p-nitrophenylphosphate + H_2O $\xrightarrow{ALP}$ phosphate + p-nitrophenol

SAMPLE[2]

Serum or heparinized plasma.
Samples are stable for 5 days when stored at +2 to +8°C.

REAGENT COMPOSITION

Contents		Concentrations in the Test
R1a.	**Buffer**	
	Diethanolamine buffer	1 mol/l, pH 9.8
	$MgCl_2$	0.5 mmol/l
R1b.	**Substrate**	
	p-nitrophenylphosphate	10 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.

Solution R1a contains diethanolamine which may cause serious damage to eyes and which is harmful if swallowed. Avoid ingestion and wear suitable eye protection.

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

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

R1b. Substrate
Reconstitute one vial of Substrate R1b with the appropriate volume of Buffer R1a:-
3 ml for the **20 x 3 ml** kit (AP 542)
10 ml for the **10 x 10 ml** kit (AP 307)
Stable for 30 days at +2 to +8°C or 3 days at +15 to +25°C.

MATERIALS PROVIDED

Buffer
Substrate

MATERIALS REQUIRED BUT NOT PROVIDED

Assayed Multi-sera 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 ALP in the Run Test screen and carry out a water blank as instructed.

Pipette into a test tube:

Sample	0.01 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:	Hg 405 nm
Cuvette:	1 cm light path
Temperature:	25°C, 30°C, 37°C
Measurement:	against air

Pipette into cuvette:	Macro	Semi-Micro	Micro
Sample	0.05 ml	0.02 ml	0.01 ml
Reagent (25°C, 30°C, 37°C)	3.00 ml	1.00 ml	0.50 ml

Mix, read initial absorbance and start timer simultaneously. Read again after 1, 2 and 3 min.

MANUAL CALCULATION

To calculate the ALP activity use the following formulae:

U/l = 3300 x ΔA 405 nm/min MACRO
U/l = 2760 x ΔA 405 nm/min SEMI-MICRO
U/l = 2760 x ΔA 405 nm/min MICRO

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QUALITY CONTROL

Randox Assayed Multi-sera, 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 Services, Northern Ireland +44 (0) 28 94451070.

INTERFERENCE

Avoid haemolysis as it interferes with the assay.
The following analytes were tested up to the following levels and were found not to interfere:

Bilirubin	300 µmol/l (17 mg/dl)
Intralipid®	2%
Triglycerides	22.75 mmol/l (2010 mg/dl)
Haemoglobin	1 g/l (100 mg/dl)

NORMAL VALUES IN SERUM

	25°C	30°C	37°C
Men/women	60-170 U/l	73-207 U/l	98-279 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 a RX **monza** analyser running at a temperature of 37°C.

SENSITIVITY

The minimum detectable concentration of ALP with an acceptable level of precision was determined as 49.9 U/l.

LINEARITY

This method is linear up to 1609 U/l. If the sample concentration exceeds this value, dilute the sample 1+9 with 0.9% NaCl solution and reassay. Multiply the result by 10.

PRECISION

Intra Assay

	Level 2	Level 3
Mean (U/l)	262	486
SD	8.11	9.49
CV(%)	3.10	1.95
n	20	20

Inter Assay

	Level 2	Level 3
Mean (U/l)	262	486
SD	11.59	17.01
CV(%)	4.43	3.50
n	20	20

CORRELATION

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

Y = 1.076X - 14.5
and a correlation coefficient of r = 0.9975

43 patient samples were analyzed spanning the range 52 to 965 U/l.

REFERENCES

1. Rec. GSCC (DGKC); J. Clin. Chem. Clin. Biochem. 1972; **10**: 182.
2. Englehardt A., *et al*, Aerztl Labor 1970 **16** 42.

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