

Liquid Reagents – ready to use

ALKALINE PHOSPHATASE

mod. IFCC

(2 Reagents)

Diagnostic reagent for quantitative in vitro determination of alkaline phosphatase (ALP) in human serum or plasma on photometric systems.

Ref.No.	Kit Size	Content
DIA010012	5 x 25 ml	4x25 ml R1+1x25 ml R2
DIA010013	5 x 50 ml	4x50 ml R1+1x50 ml R2
DIA010014	5 x 100 ml	4x100 ml R1+1x100 ml R2

Additionally offered:

DIA040012	1 x 3 mL	Diacal Auto (Calibrator)
DIA030012	1 x 5 mL	Diacon N (Control Normal)
DIA030022	1 x 5 mL	Diacon P (Control Abnormal)

TEST PARAMETERS

Method:	Colorimetric, Kinetic, Increasing Reaction, mod. IFCC
Wavelength:	405 nm (400 – 420 nm)
Temperature:	37°C
Sample:	Serum, heparin plasma
Linearity:	up to 4000 U/L (on Hitachi 911)
Sensitivity:	The lower limit of detection is 2 U/L

TEST PRINCIPE

p-Nitro-phenylphosphate + H₂O $\xrightarrow{\text{Alkaline phosphatase}}$

p-Nitrophenol + Phosphate

Under alkaline condition, colorless p-nitrophenol is converted to 4-nitrophenoxide, which develops a very intense yellow color.

Its intensity is proportional to the activity of alkaline phosphatase in the sample.

REAGENT COMPOSITION

COMPONENTS	FINAL CONCENTRATION
Reagent 1:	
2-Amino-2-Methyl-1-Propanol	0.90 mol/L
pH 10.4	
Magnesium Acetate	1.6 mmol/L
Zinc sulphate	0.4 mmol/L
HEDTA	2.0 mmol/L
Reagent 2:	
p-Nitrophenylphosphate	16.0 mmol/L

REAGENT PREPARATION

Substrate Start:

Reagents are ready for use.

Sample Start:

Mix 4 parts of Reagent 1 with 1 part of Reagent 2.
(= Working Reagent)

REAGENT STABILITY AND STORAGE

Conditions: protect from light (Reagent 2!)
close immediately after use
do not freeze the reagents!

Substrate Start:

Storage: at 2 – 8°C

Stability: up to the expiration date

Sample Start (Working Reagent):

Stability: at 2 – 8°C 4 weeks

at 15 – 25°C 5 days

Maximum allowable absorbance of the Working Reagent measured at 405 nm against water as reference is 1.0.

SAMPLE STABILITY AND STORAGE

Stability: at 4 – 8 °C 7 days

at - 20 °C 2 months

Loss of activity: at 15 – 25 °C within 2 – 3 days < 10%

Discard contaminated specimens.

INTERFERING SUBSTANCES

no interference up to:

ascorbic acid 30 mg/dl

conjugated bilirubin 60 mg/dl

unconjugated bilirubin 25 mg/dl

triglycerides 2000 mg/dl

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

Substrate Start 37°C

Pipette into test tube:	Blank:	Sample:
Reagent 1	1000 µl	1000 µl
Sample		20 µl
Dist. water	20 µl	
Mix. Incubate for approximately 1 minute. Then add:		
Reagent 2	250 µl	250 µl
Mix. Read initial absorbance after 1 minute and start a timer.		
Read absorbance again after exactly 1, 2 and 3 min.		

Sample Start 37°C

Pipette into test tubes	Blank:	Sample:
Working Reagent	1000 µl	1000 µl
Sample		20 µl
Dist. water	20 µl	
Mix. Read initial absorbance after 1 minute and start a timer.		
Read absorbance again after exactly 1, 2 and 3 min.		

CALCULATION (light path 1 cm)

$\Delta A/\text{min} = [\Delta A/\text{min sample}] - [\Delta A/\text{min blank}]$

Alkaline Phosphatase (U/L) = $\Delta A/\text{min} \times \text{Factor}$

FACTORS (37°C)

Substrate Start: 3433

Sample Start: 2757

UNIT CONVERSION

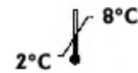
U/L x 0.01667 = µkat/L

REFERENCE RANGE*(U/L)

Adults:	Years	37 °C
Females	20-50	42–98
Females	> 60	53–141
Males	20-50	53-128
Males	> 60	56–119
Age Children:	37 °C	
	Females	Males
1–30 days	48–406	75–319
1 month–1year	124–341	82–383
1–3 year(s)	108-317	104–345
4–6 years	96–297	93–309
7–9 years	69–325	86–315
10–12 years	51–332	42–362
13–15 years	50–162	74–390



16–18 years	47-119	52–171
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* It is recommended that each laboratory establishes its own normal range.

PERFORMANCE CHARACTERISTICS

LINEARITY

The test has been developed to determine alkaline phosphatase activities which correspond to a maximal $\Delta A/\text{min}$ of 0.25. If the value is exceeded the sample should be diluted 1+9 with NaCl solution (9 g/L sodium chloride in dist. water) and results multiplied by 10.

PRECISION

Intra-assay n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	68.6	0.58	0.85
Sample 2	107	0.71	0.67
Sample 3	243	0.97	0.40

Inter-assay n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	69.2	1.37	1.99
Sample 2	104	1.22	1.08
Sample 3	238	2.40	1.01

METHOD COMPARISON

A comparison between Diagnostica Alkaline Phosphatase (y) and a commercially available test (x) using 104 samples gave following results: $y = 1.01 x + 1.51 \text{ U/l}$; $r = 0.999$.

CALIBRATION

The use of an Alkaline Phosphatase Calibrator is optional. We recommend the Diagnostica multi calibration serum **Diacal Auto**. This method is traceable to the molar extinction coefficient. The use of an ALP Calibrator is optional. We recommend:

DIA040012	1 x 3 ml	DIACAL	Assayed Multi Calibration Serum
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QUALITY CONTROL

All control sera with ALP values determined by this method can be used. We recommend:

DIA030012	1 x 5 ml	DIACON N	Assayed Control Serum Normal
DIA030022	1 x 5 ml	DIACON P	Assayed Control Serum Abnormal

AUTOMATION

Special adaptations for automated analyzers can be made on request.

WARNINGS AND PRECAUTIONS

1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. During reaction p-nitrophenol is produced which is poisonous when inhaled, swallowed or absorbed through skin. If the reaction mixture comes in contact with skin or mucous membranes wash copiously with water!
3. Take the necessary precautions for the use of laboratory reagents.

WASTE MANAGEMENT

Please refer to local legal requirements.

