CE IVD DIALAB

Liquid Reagents – ready to use

ALKALINE PHOSPHATASE

opt. DGKC
2 Reagents

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

REF	Cont.		
D03102B	**1 x 1.25 L**	1 x 1 L	Reagent 1
		1 x 250 mL	Reagent 2
D95560	**5 x 100 mL**	4 x 100 mL	Reagent 1
		1 x 100 mL	Reagent 2
D95561	**5 x 50 mL**	4 x 50 mL	Reagent 1
		1 x 50 mL	Reagent 2
D00568	**5 x 25 mL**	4 x 25 mL	Reagent 1
		1 x 25 mL	Reagent 2
D00569	**5 x 10 mL**	4 x 10 mL	Reagent 1
		1 x 10 mL	Reagent 2
DA0802*	**5 x 50 mL**	5 x 40 mL	Reagent 1
		5 x 10 mL	Reagent 2

Additionally offered:

D98485	5 x 3 mL	Calibrator	Diacal Auto
D98481	12 x 5 mL	Control normal	Diacon N
D98482	12 x 5 mL	Control abnormal	Diacon P

* Autolyser System Pack

TEST PARAMETERS

Method:	Colorimetric, Kinetic, Increasing Reaction, optimized DGKC
Wavelength:	405 nm (400 – 420 nm)
Temperature:	37°C
Sample:	Serum, heparin plasma
Linearity:	up to 4500 U/L (on Hitachi 911)
Sensitivity:	The lower limit of detection is 3 U/L

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION
Reagent 1:	
Diethanolamin, pH 9.8	1.2 mol/L
Magnesium chloride	0.6 mmol/L
Reagent 2:	
p-Nitrophenylphosphate	50 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
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
The working reagent must be protected from light!

SAMPLE STABILITY AND STORAGE [5]

Stability:	at 20 – 25 °C	7 days
	at 4 – 8 °C	7 days
	at - 20 °C	2 months

Discard contaminated specimens.

INTERFERING SUBSTANCES

no interference up to:

ascorbic acid	30 mg/dL
bilirubin	40 mg/dL
hemoglobin	150 mg/dL
triglycerides	2000 mg/dL

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

Substrate Start

Pipette into test tubes	37°C
Reagent 1	1000 µL
Sample	20 µL
Mix. Incubate for approximately 1 minute. Then add:	
Reagent 2	250 µL
Mix. Read initial absorbance against air after 1 minute and start a timer. Read absorbance again after exactly 1,2 and 3 min. Determine ΔA/min. during the linear part of the assay.	

Sample Start

Pipette into test tubes	37°C
Working Reagent	1000 µL
Sample	20 µL
Mix. Read initial absorbance against air after 1 minute and start a timer. Read absorbance again after exactly 1, 2 and 3 min. Determine ΔA/min. during the linear part of the assay.	

CALCULATION (light path 1 cm)

Alkaline Phosphatase (U/L) = ΔA/min x Factor

Factors (37°C):

Substrate Start: 3433
Sample Start: 2757

UNIT CONVERSION

U/L x 0,01667 = µkatal/L

REFERENCE RANGE [4] * [U/L]

	Year(s)	37 °C
Adults:		< 258
Children:	1–12 year(s)	< 727
Females	13-17 years	< 448
Males	13-17 years	< 935

* Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

TEST PRINCIPLE

p-Nitro-phenylphosphate + H_2O $\xrightarrow{\text{Alkaline phosphatase}}$
p-Nitrophenol + Phosphate

Under alkaline condition, colorless p-nitrophenol is converted to 4-nitrophenoxide, which develops a very intense yellow color.
Increase of absorbance is proportional to the activity of alkaline phosphatase in the sample.

PERFORMANCE CHARACTERISTICS

LINEARITY

The test has been developed to determine alkaline phosphatase activities which correspond to a maximal ΔA/min of 0.25.
If the value is exceeded the sample should be diluted with NaCl solution (9 g/L) and the result multiplied by the dilution factor.

PRECISION

Intra-assay n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	114	1.71	1.50
Sample 2	222	2.05	0.92
Sample 3	275	2.91	1.06

Inter-assay n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	120	1.93	1.60
Sample 2	223	1.89	0.85
Sample 3	279	2.36	0.85

METHOD COMPARISON

A comparison between Dialab Alkaline phosphatase DGKC (y) and a commercially available test (x) using 78 samples gave following results:
y = 0.98 x – 2.21 U/L; r= 0.999.

QUALITY CONTROL

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

REF	Cont.		
D98481	12 x 5 mL	**DIACON N**	Assayed Control Serum Normal
D98482	12 x 5 mL	**DIACON P**	Assayed Control Serum Abnormal

CALIBRATION

The use of an Alkaline Phosphatase Calibrator is optional.
We recommend:

REF	Cont.		
D98485	5 x 3 mL	**DIACAL AUTO**	Assayed Multi Calibration Serum

AUTOMATION

Special adaptations for automated analyzers can be made on request.

WARNINGS AND PRECAUTIONS

1. Reagent 1 is harmful: Xn
   R41: Risk of serious damage to eyes.
   R48/22: Harmful: danger of serious damage to health by prolonged exposure if swallowed
   S2: Keep out of the reach of children.
   S13: Keep away from food, drink and animal feedingstuffs.
   S25: Avoid contact with eyes.
   S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
   S39: Wear eye/face protection
   S46: If swallowed, seek medical advice immediately and show container or label
2. Reagent 2 contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
3. 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!
4. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.

WASTE MANAGEMENT

Please refer to local legal requirements

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4. Fischbach F, Zawta B. Age-dependent reference limits of several enzymes in plasma at different measuring temperatures. Klin Lab 1992;38:555-61.
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