ALP DGKC
Multi-Purpose (MPR)
Liquid Reagent

Glenbio
Diagnostics & Research Laboratory Solutions

KIT SPECIFICATIONS:

Cat. No.	Quantity	Reagent	Storage
GL701AP	10 x 15 ml	ALP DGKC - 1	2-8°C
	2 x 15 ml	ALP DGKC - 2	
GL711AP	5 x 50 ml	ALP DGKC - 1	
	1 x 50 ml	ALP DGKC - 2	

INTENDED USE:

In Vitro Diagnostic reagent pack for the quantitative determination of Alkaline Phosphatase (ALP) in serum and plasma, based upon the recommendations of DGKC, on automated and semi-automated analysers.

SUMMARY AND EXPLANATION: [1]

Alkaline Phosphatase catalyses the hydrolysis of naturally occurring and synthetic substrates. The natural substrates upon which they act in the body are not known. The enzyme consists of 4 structural genotypes and is present in a variety of tissues including bone, liver, intestine, placenta, kidney and spleen.

Measurements of alkaline phosphatase are used in the diagnosis of hepatobilary disease and bone disease associated with increased osteoblastic activity. Due to the increased osteoblastic activity associated with bone growth in children and juveniles, reference ranges vary very significantly with age groups.

PRINCIPLE OF THE TEST: [1]

This procedure has been formulated in accordance with the kinetic determination of the alkaline phosphatase.

p-Nitrophenol-Phosphate + H_2O $\xrightarrow{ALP}$ p-Nitrophenol- + Phosphate

WARNINGS AND PRECAUTIONS:

For In Vitro Diagnostics Use Only – For Professional Use Only
Carefully read instructions for use. Deviations from this procedure may alter performance of the assay.

Components Colour and Appearance:
Reagent 1: Clear, colourless liquid.
Reagent 2: Clear, pale yellow liquid. (Tends to darken with age but this has no effect on the assay[3]).
Any significant changes from the above could indicate that the assay might be compromised. Refer to Laboratory's QC program for actions to be taken. In case of serious damage to the bottle and/or cap, resulting in product leakage and/or contamination, do not use the reagent pack and contact your distributor.

Safety precautions:
CAUTION: Take all necessary precautions required when handling laboratory reagents. Contains Sodium Azide. Material Safety Data Sheet is available upon request.

Label Elements:

DANGER

H315 - Causes skin irritation.

Precautionary Statements:
P264 – Wash thoroughly after handling.
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 – Immediately call a POISON CENTER or doctor/physician.

Handling precautions:
- Do not use components past the expiry date stated on the Bottles.
- Do not Freeze Reagents.
- Do not use components for any purpose other than described in the "Intended Use" section.
- Do not interchange caps among components as contamination may occur and compromise test results.
- Refer to local legal requirements for safe waste disposal.

INSTRUMENTS:

Instrument applications are available upon request.

COMPONENT COMPOSITION:

Component	Ingredients	Concentration in Tests
Reagent 1	Diethanolamine Buffer pH 9.8	1 mol/l
	Magnesium Chloride	0.51 mmol/l
Reagent 2	p-Nitrophenyl-Phosphate	61.0 mmol/l
	PRESERVATIVES	---

REAGENT PREPARATION AND STABILITY:

Before use, mix each reagent by gently inverting each bottle.
If stored and handled properly, unopened components are stable until the expiry date stated on the label
Monoreagent procedure: Add 1 volume of Reagent 2 to 5 Volumes of Reagent 1.
Working reagent is stable 4 weeks at 2-8°C.
Bireagent procedure: Liquid reagent 1 and 2 are ready for use.
Once open, components are stable until expiry date on label if store and handled properly.

TYPE OF SPECIMEN: [1]

Use serum or heparin plasma, free of haemolysis, as specimen. Do Not Use Citrate, Oxalate and EDTA.

It is recommended to follow CLSI procedures (or similar standardised conditions) regarding specimen handling. Specimen should be collected in an appropriate sample container, with proper specimen identification. Serum/plasma should be separated from cells within 2 hours after collection.

Stability: up to 7 days at 2-8°C[4].

TEST PROCEDURE:

Materials required but not supplied:

Description	Catalog. No.	Description	Catalog. No.
General Chemistry Calibrator	GL983	Photometer	N/A
General Chemistry Control Level 1	GL922	General Laboratory Equipment	N/A
General Chemistry Control Level 2	GL932		

Assay procedure:
Wavelength: λ: 405 nm (410 nm)
Temperature: 25°C (30°C or 37°C)
Optical path: 1 cm light path.

MONOREAGENT PROCEDURE:	Blank	Calibrator	Sample
Working reagent	1000 µl	1000 µl	1000 µl
Sample	----	----	20 µl
Calibrator	----	20 µl	----
Gently mix sample and reagent and incubate for 1 minute, then read absorbance after a further 1, 2 and 3 minutes. Calculate the absorbance. Calculate the absorbance change (ΔOD/min) per minute.			

BIREAGENT PROCEDURE:	Blank	Calibrator	Sample
Reagent 1	1000 µl	1000 µl	1000 µl
Sample	----	----	6 µl
Calibrator	----	20 µl	----
Gently mix and Incubate at 37°C for 2 minutes			
Reagent 2	200 µl	200 µl	200 µl
Gently mix and incubate for 1 minute, then read absorbance after a further 1, 2, 3 and 4 minutes. Calculate the absorbance. Calculate the absorbance change (ΔOD/min) per minute.			

Enzyme Calibration:
Using recommended Calibrator, calibrate the assay:
- Daily.
- When using a new reagent kit or changing lot number.
- Following preventive maintenance or replacement of a critical part of the photometer used.
- When Quality Controls are out of range.

Quality Control:
All clinical laboratories should establish an Internal Quality Control program. Verify instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to the Laboratory's QC program.
Controls should be assayed:
- Prior reporting patient results.
- Following any maintenance procedure on the photometer used.
- At intervals established by the Laboratory's Q.C. Programme.

CALCULATION:

405 nm: U/l = ΔOD/min x 2757
410 nm: U/l = ΔOD/min x 2014
*The above factors should be validated using General Chemistry Calibrator (GL973).
(Conversion factor: Qty in µKat/l = Qty in U/l x 0.0167).

EXPECTED VALUES[4, 5]

Reference range by Rosalki[4]	U/l at 25°C	µKat/l at 25°C	U/l at 30°C*	µKat/l at 30°C*	U/l at 37° C*	µKat/l at 37° C*
Men	< 180	< 3.06	< 220	< 3.74	< 270	< 4.59
Women	<160	< 2.72	< 195	< 3.32	< 240	< 4.08

*Calculated values. †Temperature conversion factor from 25°C to 30°C: 1.24 and to 37°C: 1.52

Each laboratory should establish its own reference range. ALP results should always be reviewed with the patient's medical examination and history.

PERFORMANCE CHARACTERISTICS:

Performance results can vary with the instrument used. Data obtained in each individual laboratory may differ from these values.

Linearity:
Linear up to 1524 U/l (25.9 µKat/l).
For samples with a higher concentration, dilute 1:1 with 0.9% NaCl (9g/l) and re-assay. Multiply result by 2.

Interfering substances:

Bilirubin (mixed isomers):	Less than 10% interference up to 600 µmol/l Bilirubin
Haemolysis:	Less than 10% interference up to 5 g/l Haemoglobin.
Lipemia:	Less than 10% interference up to 5 g/l Intralipid.

Sensitivity:
The Lowest Detectable Level was estimated at 4.0 U/l (0.068 µKat/l).

Precision:

Within Run N = 20	Mean (U/l)	SD	% CV	Between Run N = 20	Mean (U/l)	SD	% CV
Level 1	171	1.37	0.78	Level 1	176	5.66	3.21
Level 2	454	2.99	0.66	Level 2	441	9.76	2.22

Method Comparison:
Using 50 samples, a comparison, between this ALP test (y) and another commercially available test (x), gave the following results:

y = 1.139x – 6.453	r =0.998	Sample range: 48 to 577 U/l

BIBLIOGRAPHY:

1. DGKC STD Method J. Clin. Chem. 1972; 10:290 and Burtis CA, Ashwood ER. Tietz Fund. Of Clin. Chem. 5th ed. 30:54, 366-369.
2. Guder WG, Narayanan S, Wisser H, Zawta B. List of Analytes Pre-analytical Variables. Brochure in: Samples: From the patient to the Laboratory. Darmstadt: GIT Verlag, 1996.
3. Rosalki SB, Foo AY, Burlina A, et al. Multicenter Evaluation of Iso-ALP TEST Kit for Measurement of Bone Alkaline Phosphatase Activity in Serum and Plasma. Clin. Chem. 1993; 39:648-652.
4. Fishchback F, Zawta B. Age Dependent reference limits of several enzymes in plasma at different measuring temperatures. Klin Lab 1992; 38: 555.

SYMBOLS:

The following symbols are used in the labelling of Glenbio systems:

IVD	In Vitro Diagnostics	REF	Catalogue No
LOT	Batch Code	CONT	Content
REAG	Reagent	Ab	Antibody
CAL	Calibrator	SUBS	Substrate
BUF	Buffer	STD	Aqueous Standard

CE Mark - Device complies with the Directives 98/79/EC

Storage temperature → Reconstitute with

Expiry Date (Last day of the month) — Manufactured By

Biological risk — Consult Instruction for Use

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