Alkaline Phosphatase (ALP)
(DGKC/DEA Method)

- **Kit Specifications:**

	Reagent/Quantity	Storage
Cat. No.: **AP0011**	R.1: 4 x 50 ml R.2 1 x 50 total 250 ml	2-8°C
Cat. No.: **AP0017**	R.1: 4 x 50 ml R.2 1 x 50 total 250 ml	2-8°C

- **Intended Use:**

In Vitro Diagnostic reagent pack for the quantitative determination of Alkaline phosphatase in human serum and plasma on automated and semi-automated photometric systems.

- **Summary and Explanation:**

Alkaline phosphatase is the most popular enzyme which can find in most of the tissue in the human body especially in bone, liver, placenta, bowl and kidney. This enzyme is hydrolytic and the optimal activity is in high pH condition in alkaline base. In the other tissue such as thymus gland, lung and testicle was found. The amount of this enzyme in serum is increased in childhood, pregnancy, liver and bone destruction. Also, this enzyme is find in enclosed vascular biliary, liver inflammation, cholestasis, obstructive jaundice, hepatitis infection, bone diseases and in the high activity of osteoblast. The high amount of this enzyme is in Paget, rickets, osteomalacia and hyper parathyrohidism. The decrease amount of ALP is found in deficiency of vitamin C and cretinism.

Therefore, the increase and decrease amount of this enzyme in plasma is critical in clinical diagnosis.

- **Principle of the Method:**

This method based on Alkaline phosphatase exist on serum in basic background by hydrolysis of p-Nitrophenyl Phosphate in 10.4 pH catalyze and release phosphate and p-Nitrophenol. The intensity of the color will have measured on 405 Nano meter wavelength which is correlate with the amount of ALP on the sample.

p-Nitrophenylphosphate + H2O $\xrightarrow{ALP}$ p-Nitrophenol + Phosphate

- **Reagent Preparation and Stability:**

Reagents is ready for use.

Before use, mix reagent by gently inverting each bottle.

Reagent is stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use. The stability of mixture at 2-8°C for 30 days and at 15-25°C for 10 days.

Do not use reagents over the expiration date.

Do not freeze and protect from light.

The reagents mixing should follow the order 1 volume of R2 and 4 volumes of R1.

R1 is transparent and colorless but R2 is transparent and light yellow.

Waste Management:

Refer to local legal requirements for chemical disposal regulations.

Warning: Handle waste as potentially biohazardous material.

Dispose of waste according to accepted laboratory instructions and procedures.

- **Warnings and Precautions:**

For In Vitro Diagnostics Use Only.

For Professional Use Only.

Carefully read instructions for use.

In case of serious damage to the bottle or cap, resulting in product leakage or contamination, do not use the reagent pack and contact your distributor.

Take all necessary precautions required when handling laboratory reagents.

Do not use components past the expiry date stated on the Bottles.

Do not interchange caps among components as contamination may occur and compromise test results. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.

Any serious incident related to the product must be reported to the manufacturer and the competent authority of the Member State where the user and/or patient is located.

- **Type of Specimen:**

Use fresh serum, non-hemolysis and/or plasma heparinized but do not use EDTA, citrate and oxalate.

It is recommended to follow the procedures (or similar standardized conditions) regarding specimen handling. Specimen should be collected in an appropriate sample container, with proper specimen identification.

Stability:

The stability of ALP at -20°C will be 60 Days. Immediately serum and plasma should be collected after separation from cells. In dark, avoid sunlight direct, avoid any contamination. The decreased ALP activity at 20 to 25°C will be less than 10 percent.

- **Materials Required but not Supplied:**

General chemistry calibrator from TKS or other valid calibrators.

General chemistry control Level 1 & 2 from TKS or other valid controls.

Saline solution 0.9 % NaCl

General laboratory equipments.

- **Notes:**

Carefully read instructions for use.

It is recommended to use disposable material. If glassware is used the material should be scrupulously cleaned with hydrochloric acid 1 N and then thoroughly rinsed it with distilled water.

Use clean disposable pipette tips for its dispensation.

Disposable and glassware material used must be free of metals, ions and detergents.

- **Performance Characteristics:**

Performance results can vary with the instrument used.

Data obtained in each individual laboratory may differ from these values.

Maximum determination in this assay is 1200U/L

LOD: 3 U/L

For samples with a higher concentration (1200U/L), dilute 1:9 with 0.9 % NaCl and re-assay. Multiply result by 10.

Precision:

Intra Assay-Within run ALP

Sample	n	Mean (µg/L)	SD (µg/L)	CV (%)
1	20	174	0.72	0.41
2	20	443	1.56	0.35

Inter Assay-Between run ALP

Sample	n	Mean (µg/L)	SD (µg/L)	CV (%)
1	20	175	6.88	3.93
2	20	434	11.93	2.75

- **Accuracy:**

Results obtained using BIOMEDIC reagents (y) did not show systematic differences when compared with other commercial reagents (x).

Correlation coefficient (r): 0.999

Regression equation: Y = 1.025 (X) - 1.105 U/L

The results of the performance characteristics depend on the analyzer used.

- **Interfering Substances:**

Fluoride, oxalate, citrate and EDTA suppress the activity of this enzyme. So the anti-coagulant should not use. The ALP is high in hemolysis and the red blood cells make interference in this assay.

- **Reference Values:**

Age	At 25°C	At 30°C	At 37°C
Children 1-14 years	**<400U/L**	**<480U/L**	**<645U/L**
Adults	**60-170 U/L**	**73-207 U/L**	**98-279 U/L**

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

- **Assay Procedure:**

Allow reagents to reach working temperature before using.

A proportional variation of the reaction volumes indicated does not change the result

Assay conditions:

405nm	**Wavelengths**
37 °C	**Incubation Temperature**

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1 cm	**Cuvette**

Adjust the instrument to zero with distilled water.

Control/Sample/Calibrator	**Blank**	
1000 µl	1000 µl	**R1**
20 µl	-	**Control/Sample/Calibrator**
Gently mix and incubate at 37°C for5 minutes. Then added R2. The absorption of sample and calibrator against blank.		

Control/Calibrator/Sample	**Blank**	
250µl	250µl	**R2**
Gently mix and incubate for 1 minute at 37°C. then measure the absorbance from sample and A calibrator. Turn on the counter 1, 2 and 3 minutes before start absorbance. **ΔOD/min**		

Control/Calibrator/Sample	**Blank**	**Single Reference**
1200 µl	**1200 µl**	**Ref ready to use**
20 µl	-	**Control/Calibrator/Sample**

Gently mix and incubate for 1 minute at 37°C. then measure the absorbance from sample and A calibrator. Turn on the counter 1, 2 and 3 minutes before start absorbance. **ΔOD/min.**

- **Calculations:**

ALP (U/L) = Abs. Sample/min x Cal.Conc. (U/L)
Abs. Calibrator/min

ALP (U/L) = ΔA/min x 3300

TOSE'E KIMIA SA'ADAT has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

- **References:**

1-Wenger C. et al. Alkaline phosphatase. Kaplan A et al. Clin Chem the C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1094-1098.
2-Rosalki S et al. Clin Chem 1993; 39/4: 648-652.
3-Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
4-Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
5-Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
6-Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

- **Symbols:**

The following symbols are used in the labelling of TOSE'E KIMIA SA'ADAT systems:

IVD	In Vitro Diagnostics		Contains sufficient for <n> tests
LOT	Batch Code		Temperature limit
REF	Catalogue No.		Consult instruction for use
	Expiry Date		Caution
	Date of Manufacture		Keep dry
	Manufactured by		This way up
	Biological Risks		Keep away from sunlight

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ISO 13485:2016
ISO 9001:2015

IVD

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