（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K1304-M

Specification：48T (46 samples)/96T (94 samples)

Measuring instrument：Microplate reader (400 - 410 nm)

Detection range：6.09-4500 U/L

Elabscience® Alkaline Phosphatase (ALP) Activity Colorimetric Assay Kit (Method of NPP-AMP)

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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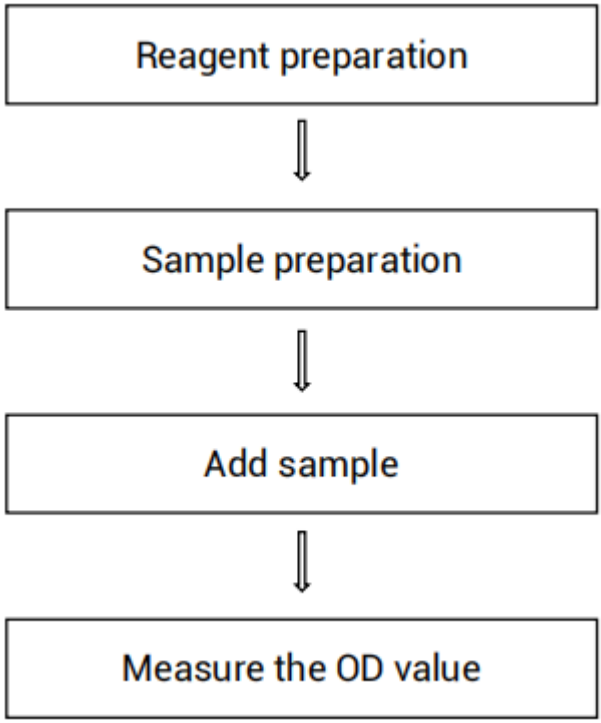
Assay summary



Intended use

This kit can measure alkaline phosphatase (ALP) activity in serum, plasma, animal tissue and cell samples.

Detection principle

Using 4-Nitrophenyl phosphate (4-NPP) as the substrate and 2-Amino-2-methyl-1-propanol (AMP) as the receptor substance for the phosphate group, alkaline phosphatase (ALP) catalyzes the reaction to generate free 4-nitrophenol (4-NP). The latter converts into a quinone structure in an alkaline solution and exhibits a darker yellow color. The activity of ALP in the sample can be calculated based on the rate of increase in absorbance at 405 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	12 mL × 1 vial	24 mL ×1 vial	2-8°C, 12 months
Reagent 2	Substrate Solution	3 mL × 1 vial	6 mL × 1 vial	2-8°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (400-410 nm, optimum wavelength: 405 nm),

Incubator (37°C)

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

Take an appropriate amount of reagent according to the detection requirements and incubate it to 37°C before use.

Sample preparation

① **Sample preparation**

Serum and plasma samples: detect directly.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. The supernatant should be used up within 8 h.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell samples:

① Harvest the number of cells needed for each assay (initial recommendation 2×10^6 cells).

② Homogenize 2×10^6 cells in 200 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4°C.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. The supernatant should be used up within 8 h.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② **Dilution of sample**

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
Rats serum	1
Horse serum	1
Human plasma	1
Rabbit serum	1
Porcine serum	1
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
2×10^6 Hela cells	1
2×10^6 HepG2 cells	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

If the ΔA value of the sample is not significant (less than 0.005), the incubation time for the second round can be extended to 5 min. Correspondingly, the reaction time in the calculation formula should be modified to 5 min.

Operating steps

① Blank well: Add 5 μL of normal saline (0.9% NaCl) to the wells.
Sample well: Add 5 μL of samples to the wells.

② Add 200 μL of buffer solution to each well.

③ Mix fully with microplate reader for 5 s, incubate at 37°C for 5 min.

④ Add 50 μL of substrate solution to each well.

⑤ Mix fully with microplate reader for 5 s, measure the OD value of each well at 405 nm with microplate reader, as A_1.

⑥ Incubate at 37°C for 3 min, measure the OD value of each well at 405 nm with microplate reader, as A_2.

Calculation

The sample:

1. Serum and plasma samples:

Definition: The amount of enzyme in 1 L serum (plasma) per 1 min that catalyze decomposition of 1 μmol product at 37°C is defined as 1 unit.

$$\underset{(U/L)}{\text{ALP activity}} = (\Delta A_{sample}\text{-}\Delta A_{blank}) \div (\varepsilon \times d) \times \frac{V_{total}}{V_{sample}} \times 10^{6*} \div T \times f$$

$$= (\Delta A_{sample} - \Delta A_{blank}) \times 1218.64^{*} \times f$$

2. Tissue samples and cell samples:

Definition: The amount of enzyme in 1 g tissue or cell protein per 1 min catalyze decomposition of 1 μmol product at 37°C is defined as 1 unit.

$$\underset{(U/gprot)}{\text{ALP activity}} = (\Delta A_{sample} - \Delta A_{blank}) \div (\varepsilon \times d) \times \frac{V_{total}}{V_{sample}} \times 10\text{^}6^{*} \div T \div C_{pr} \times f$$

$$= (\Delta A_{sample} - \Delta A_{blank}) \times 1218.64^{*} \div C_{pr} \times f$$

[Note]

ΔA_{sample}: The A_2 value of sample well - the A_1 value of sample well.

ΔA_{blank}: The A_2 value of blank well - the A_1 value of blank well.

ε: The molar extinction coefficient, 18600 L/(mol·cm).

d: Optical path, 0.75 cm.

V_{total}: The volume of reaction system, 255 μL.

V_{sample}: The volume of sample, 5 μL.

10^6*: 1 mol = 1×10^6 μmol

T: Reaction time, 3 min.

f: Dilution factor of sample before test.

1218.64*: Simplified value.

Cpr: Concentration of protein in sample, gprot/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	333.3	666.7	1000.0
%CV	1.3	3.1	2.5

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	333.3	666.7	1000.0
%CV	2.6	4.1	3.5

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 97.3%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (μmol/L)	133.3	166.7	200.0
Observed Conc. (μmol/L)	133.7	159.7	192.7
Recovery rate (%)	100	96	96

Sensitivity

The analytical sensitivity of the assay is 6.09 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix Π Example Analysis

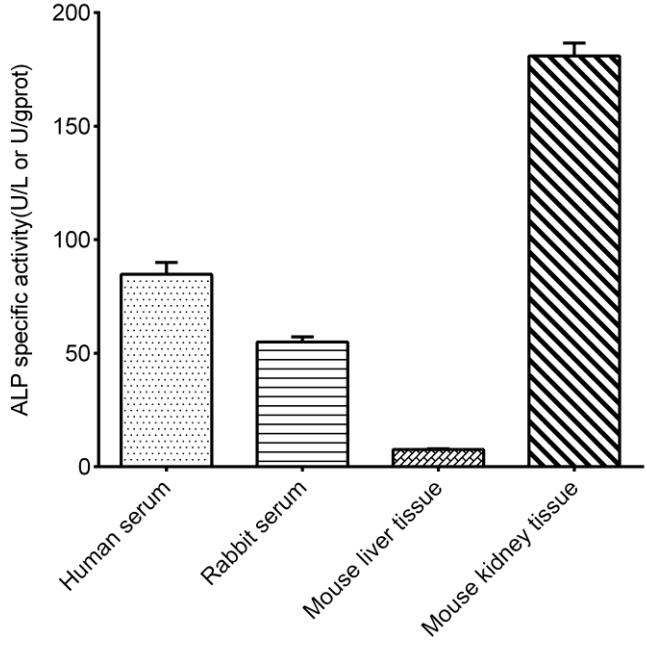
Example analysis：

Take 5 μL of human serum, and carry the assay according to the operation steps. The results are as follows:

The A_1 value of the blank well is 0.302, the A_2 value of the blank well is 0.303, ΔA_{blank} = 0.303 - 0.302 = 0.001; the A_1 value of the sample well is 0.304, the A_2 value of the sample well is 0.375, ΔA_{sample} = 0.375 - 0.304 = 0.071, and the calculation result is:

$$\text{ALP activity (U/L)} = (0.071 - 0.001) \times 1218.64 = 85.30 \text{ U/L}$$

Detect human serum, rabbit serum, 10% mouse liver tissue homogenate(the concentration of protein is 10.83 gprot/L), 10% mouse kidney tissue homogenate(the concentration of protein is 6.74 gprot/L), according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.