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

Catalog No：E-BC-K091-M

Specification：96T(80 samples)/ 96T×5 (464 samples)

Measuring instrument：Microplate reader（500-530 nm）

Detection range：0.13-50 King unit/100 mL

Elabscience®Alkaline Phosphatase (ALP) Activity Assay Kit

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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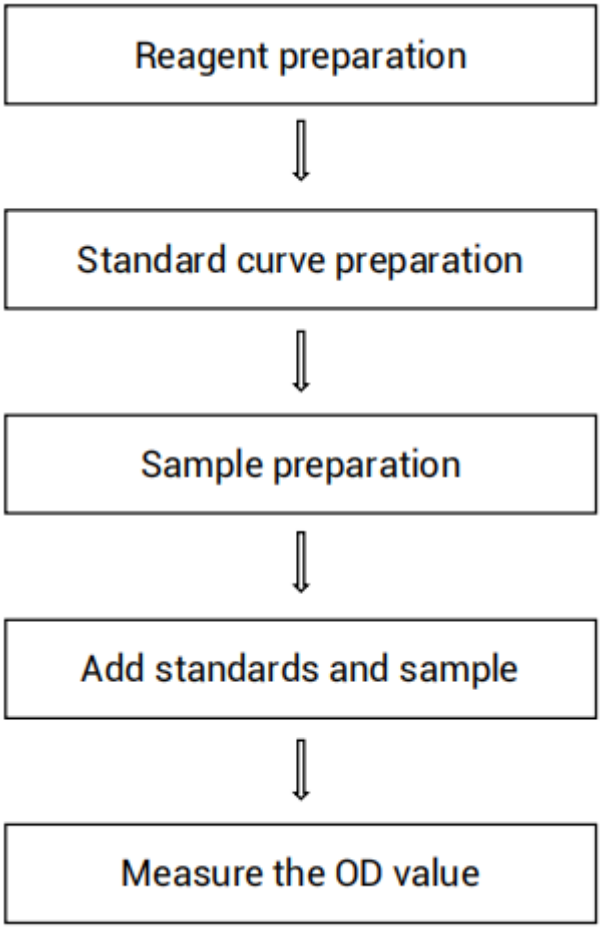
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Assay summary

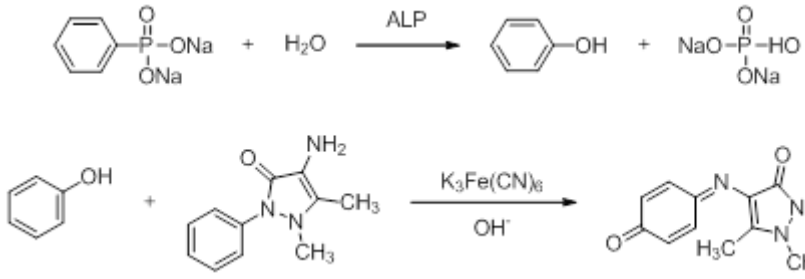


Intended use

This kit can be used to measure alkaline phosphatase (ALP) activity in serum (plasma), tissue, cells and other samples.

Detection principle

Alkaline phosphatase decompose benzene disodium phosphate to produce free phenol and phosphoric acid. Phenol react with 4-aminopyrline in alkaline solution and oxidizes with potassium ferricyanide to form red quinone derivative. The enzyme activity can be calculated indirectly by measuring the OD value.



Kit components & storage

Item	Component	Size 1 (96 T)	Size 2 (96T×5)	Storage
Reagent 1	Buffer Solution	3 mL × 1 vial	15 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 2	Substrate Solution	3 mL × 1 vial	15 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 3	Chromogenic Agent	18 mL × 1 vial	50 mL × 2 vials	2-8℃, 12 months, shading light
Reagent 4	0.5 mg/mL Phenol Standard	1.5 mL × 1 vial	5 mL × 1 vial	2-8℃, 12 months, shading light
	Microplate	96 wells	96 wells× 5	No requirement
	Plate Sealer	2 pieces	7 pieces	
	Sample Layout Sheet	1 piece	7 pieces	

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 (500-530 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of working solution：

For each well， prepare 50 μL of working solution (mix well 25 μL of buffer solution and 25 μL of substrate solution). Store at 2-8°C for 1 day protected from light.

③ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 0.5 mg/mL phenol standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 mg/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mg/mL)	**0**	**0.025**	**0.05**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**
0.5 mg/mL phenol standard (μL)	0	5	10	20	40	60	80	100
Double distilled water (μL)	100	95	90	80	60	40	20	0

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

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

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell (adherent or suspension) samples:

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

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 300-500 μL normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ 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 plasma	1
Human urine	1
Rat serum	1
Cells culture supernatant	1
10% Mouse kidney tissue homogenate	30-50
10% Mouse liver tissue homogenate	1
10% Mouse brain tissue homogenate	1
HepG2 cells	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: add 5 μL of standards with different concentrations to the corresponding wells.
Sample well: add 5 μL of sample to the corresponding wells.

② Add 50 μL of working solution and mix fully for 30 s with microplate reader.

③ Incubate at 37℃ for 15 min, then add 150 μL of chromogenic agent immediately, mix fully.

④ Measure the OD values of each well at 520 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

Definition: The amount of 1 mg phenol produced by 100 mL sample react with the substrate in 15 min is defined as 1 ALP activity unit.

$$\text{ALP activity (King unit/100 mL)} = (\Delta A\text{-}b) \div a \times V_1 \times f$$

2. Tissue and cells sample:

Definition: The amount of 1 mg phenol produced by 1 g tissue protein react with the substrate in 15 min is defined as 1 ALP activity unit.

$$\text{ALP activity (King unit/gprot)} = (\Delta A - b) \div a \div C_{pr} \times f$$

[Note]

ΔA: Absolute OD ($OD_{Sample} - OD_{Blank}$).

V_1: The volume of sample in definition, 100 mL.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, gprot/mL.

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 (King unit/100 mL)	1.40	24.60	41.50
%CV	5.4	4.9	5.0

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 (King unit/100 mL)	1.40	24.60	41.50
%CV	8.7	8.0	8.8

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 94%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mg/mL)	0.036	0.17	0.38
Observed Conc. (mg/mL)	0.0	0.2	0.4
recovery rate(%)	95	93	94

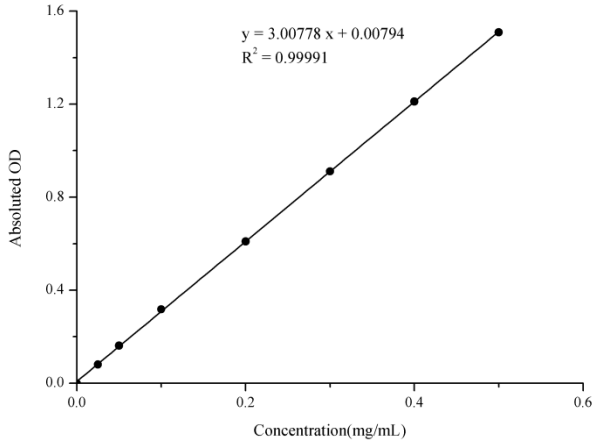
Sensitivity

The analytical sensitivity of the assay is 0.13 King unit/100 mL. 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.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (mg/mL)	**0**	**0.025**	**0.05**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**
Average OD	0.054	0.135	0.215	0.371	0.664	0.965	1.265	1.563
Absoluted OD	0	0.081	0.161	0.317	0.610	0.911	1.211	1.509



Appendix Π Example Analysis

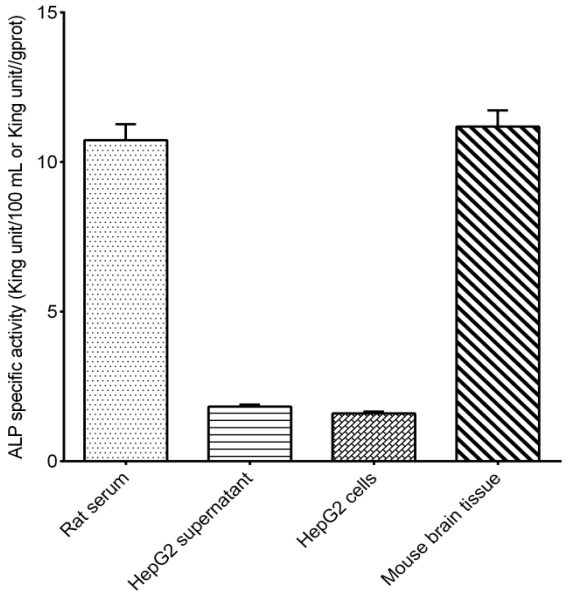
Example analysis：

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

Standard curve: y = 3.059 x + 0.0027, the average OD value of the sample well is 0.422, the average OD value of the blank well is 0.091, the concentration of protein in sample is 9.23 gprot/L, and the calculation result is:

$$\text{ALP activity (King unit/100 mL)} = (0.422 - 0.091 - 0.0027) \div 3.059 \times 100 = 10.73 \text{ King unit/100 mL}$$

Detect rat serum, 10% mouse brain tissue homogenate (the concentration of protein is 0.004 gprot/L dilute for 2 times), culture supernatant of HepG2 cells and HepG2 cells (the concentration of protein is 0.01 gprot/mL) according to the protocol, the result is as follows：



Appendix III Publications

1. Zhu N, Chen S, et al. Enhancing Glioblastoma Immunotherapy with Integrated Chimeric Antigen Receptor T Cells through the Re-Education of Tumor-Associated Microglia and Macrophages[J]. ACS nano, 2024, 18(17): 11165-11182.
2. Mo W, Liu S, Zhao X, et al. ROS scavenging nanozyme modulates immunosuppression for sensitized cancer immunotherapy[J]. Advanced Healthcare Materials, 2023, 12(21): 2300191.
3. Yuan J, Ding L, Han L, et al. Thermal/ultrasound-triggered release of liposomes loaded with Ganoderma applanatum polysaccharide from microbubbles for enhanced tumour ablation[J]. Journal of Controlled Release, 2023, 363: 84-100.
4. Wang Y, Kong B, Chen X, et al. BMSC exosome-enriched acellular fish scale scaffolds promote bone regeneration[J]. Journal of Nanobiotechnology, 2022, 20(1): 444.
5. Zeng Z, Quan C, Zhou S, et al. Gut microbiota and metabolic modulation by supplementation of polysaccharide-producing Bacillus licheniformis from Tibetan Yaks: A comprehensive multi-omics analysis[J]. International Journal of Biological Macromolecules, 2024, 254: 127808.

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.