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

Catalog No：E-BC-K091-S

Specification：100 Assays(96 samples)

Measuring instrument：Spectrophotometer（520 nm）

Detection range：0.2-55.6 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.

Table of contents

Assay summary 3

Intended use 4

Detection principle 4

Kit components & storage 4

Materials prepared by users 5

Reagent preparation 5

Sample preparation 6

The key points of the assay 7

Operating steps 8

Calculation 9

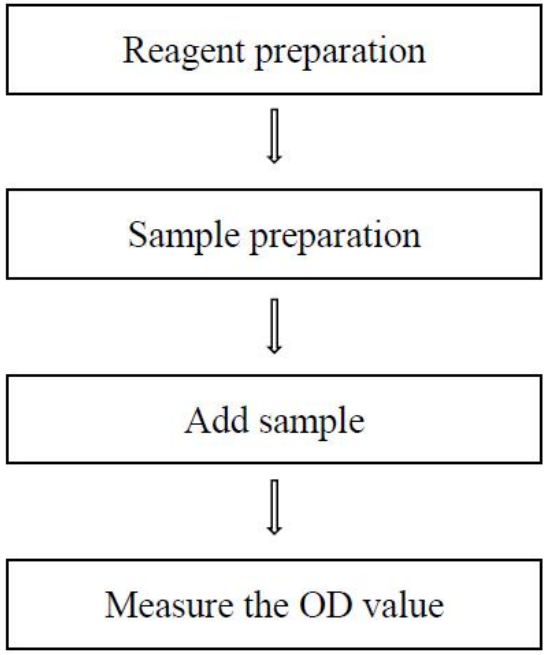
Appendix I Performance Characteristics 10

Appendix II Example Analysis 12

Appendix III Publications 13

Statement 14

Assay summary

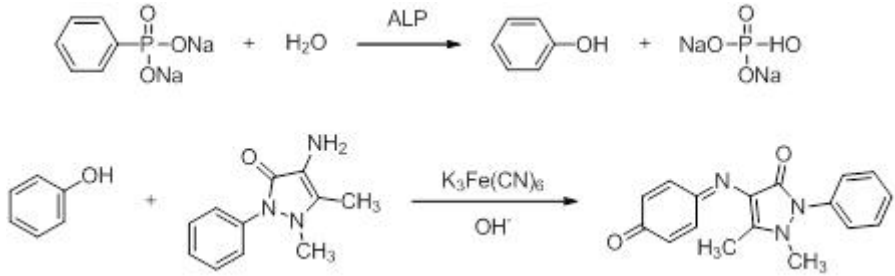


Intended use

This kit can be used for detection of ALP activity in serum, plasma, urine, tissue and cells sample.

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 (100 Assays)	Storage
Reagent 1	Buffer Solution	60 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 2	Substrate Solution	60 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 3	Chromogenic Agent	60 mL × 3 vials	2-8℃, 12 months, shading light
Reagent 4	0.5 mg/mL Phenol Standard	1.5 mL × 1 vial	2-8℃, 12 months, shading light

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:

Spectrophotometer (520 nm), Micropipettor, Incubator, Vortex mixer, Centrifuge

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 Preparation of 0.1 mg/mL phenol standard application solution：
For each well，prepare 50 μL of 0.1 mg/mL phenol standard application solution (mix well 10 μL of 0.5 mg/mL phenol standard and 40 μL of double distilled water). The 0.1 mg/mL phenol standard application solution should be prepared on spot.

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 at 4℃ 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 x g for 10 minutes at 4℃ 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 serum	1
Human urine	1
Rat serum	1
Cellular supernatant	1
10% Mouse kidney tissue homogenization	30-50
10% Mouse liver tissue homogenization	1
HePG2 cell	1
10% Mouse brain tissue homogenization	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.

The key points of the assay

Add chromogenic agent immediately after incubating at 37 ℃ for 15 min.

Operating steps

① Blank well: add 50 μL of double distilled water into a 5 mL EP tube

Standard well: add 50 μL of 0.1 mg/mL phenol standard application solution into a 5 mL EP tube.

Sample well: add 50 μL of sample into a 5 mL EP tube.

② Successively add 500 μL of buffer solution and 500 μL of substrate solution respectively and mix fully with a vortex mixer.

③ Incubate at 37℃ for 15 min, then add 1500 μL of chromogenic agent immediately, mix fully.

④ Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 520 nm with 0.5 cm optical path quartz cuvette.

Calculation

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.

$$\text{ALP activity (King unit/100 mL)} = \frac{\Delta A_1}{\Delta A_2} \times m \times \frac{V_1}{V} \times f$$

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.

$$\text{ALP activity (King unit/gprot)} = \frac{\Delta A_1}{\Delta A_2} \times m \div (C_{pr} \times V) \times f$$

[Note]

ΔA_1: $OD_{Sample} - OD_{Blank}$.

ΔA_2: $OD_{Standard} - OD_{Blank}$.

m: Phenol content of standard tube, 0.005 mg.

C_{pr}: Protein concentration of tested sample, gprot/mL.

V: The volume of sample, 0.05 mL.

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

f: Dilution factor of sample before test.

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)	2.60	28.40	42.50
%CV	2.5	2.1	1.7

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)	2.60	28.40	42.50
%CV	5.3	5.5	6.0

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 99%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (King unit/100 mL)	13.8	22.5	39.5
Observed Conc. (King unit/100 mL)	13.7	21.6	40.3
recovery rate(%)	99	96	102

Sensitivity

The analytical sensitivity of the assay is 0.2 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

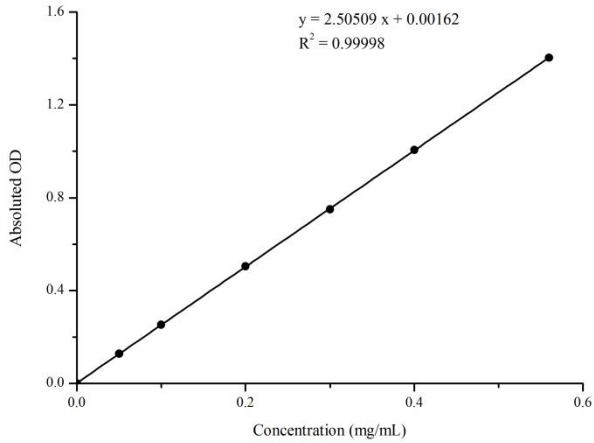
concentration.

2. Standard curve:

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

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**	**0.05**	**0.1**	**0.2**	**0.3**	**0.4**	**0.56**
Average OD	0.017	0.145	0.270	0.522	0.767	1.023	1.421
Absoluted OD	0	0.128	0.253	0.505	0.750	1.006	1.404



Appendix Π Example Analysis

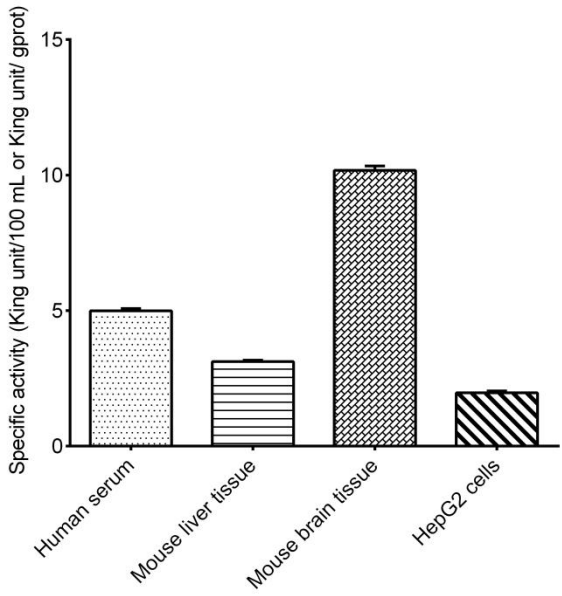
Example analysis：

Take 50 μL of rat serum, carry the assay according to the operation steps. The results are as follows:

The average OD value of the sample is 0.345, the average OD value of the blank is 0.020, the average OD value of the standard is 0.270, and the calculation result is:

$$\text{ALP activity (King unit/100 mL)} = \frac{0.345\text{-}0.020}{0.270\text{-}0.020} \times 0.005 \times \frac{100}{0.05} = 13 \text{ King unit/100 mL}$$

Detect human serum, 10% mouse liver tissue homogenate (the concentration of protein in sample is 0.013 gprot/mL), 10% mouse brain tissue homogenate (the concentration of protein in sample is 0.004 gprot/mL), HepG2 cells (the concentration of protein in sample is 0.003 gprot/mL) according to the protocol, the result is as follows：



Appendix III Publications

1. Liu C , Zhang Z , Li B ,et al.Lipid Metabolic Disorders Induced by Organophosphate Esters in Silver Carp from the Middle Reaches of the Yangtze River[J].Environmental Science & Technology: ES&T, 2024(11):58.DOI:10.1021/acs.est.3c08610.
2. Bhowmik A D , Das T , Chattopadhyay A .Chronic exposure to environmentally relevant concentration of fluoride impairs osteoblast's collagen synthesis and matrix mineralization: Involvement of epigenetic regulation in skeletal fluorosis[J].Environmental Research, 2023, 236(Part2):11.DOI:10.1016/j.envres.2023.116845.
3. Ma X, Zhang W, Chen Y, et al. Paeoniflorin inhibited GSDMD to alleviate ANIT-induced cholestasis via pyroptosis signaling pathway[J]. Phytomedicine, 2024, 134: 156021.
4. Sheng Y, Zhou J, Zhang P, et al. Effect of chiral polymers on Muse cell proliferation and differentiation[J]. Materials Today Chemistry, 2024, 42: 102425.
5. Xue X, Zhao X, Wang J, et al. Carthami flos extract against carbon tetrachloride-induced liver fibrosis via alleviating angiogenesis in mice[J]. Phytomedicine, 2023, 108: 154517.

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.