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

Catalog No：E-BC-K009-M

Specification：96T(79 samples)/500Assays(483 samples)

Measuring instrument：Microplate reader（405 nm）

Detection range：0.27-50.8 U/L

Elabscience® Alkaline Phosphatase (ALP) Activity Assay Kit (PNPP Method)

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
Tel: 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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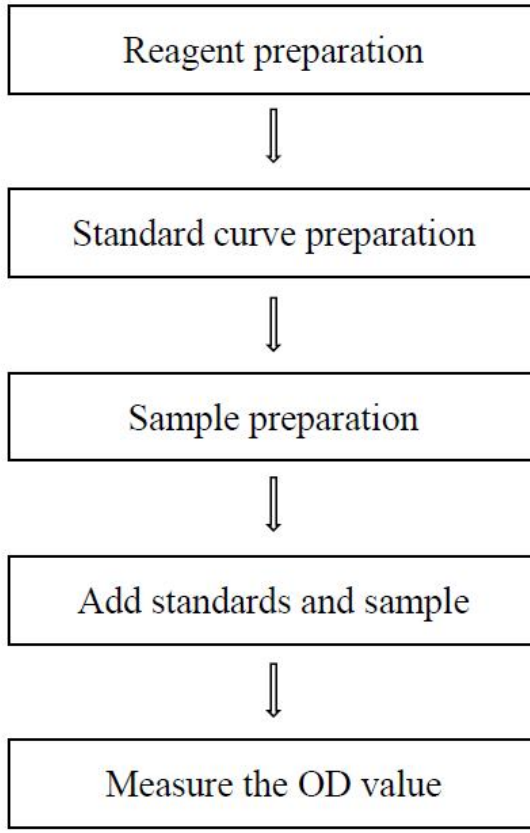
Assay summary



Intended use

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

Detection principle

Under alkaline conditions, alkaline phosphatase catalyzes the hydrolysis of p-nitrobenzene phosphate disodium to produce p-nitrophenol and phosphoric acid. Under strong alkaline conditions, p-nitrophenol is bright yellow and has a maximum absorption peak at 405 nm. Therefore, the activity of ALP can be calculated by measuring the OD value at 405 nm.

Kit components & storage

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 1	Buffer Solution	60 mL×1 vial	60 mL×5 vials	-20℃, 12 months
Reagent 2	Substrate	Powder×2 vials	Powder×10 vials	-20℃, 12 months, shading light
Reagent 3	10 mmol/L p-Nitrophenol Standard Solution	0.4 mL×1 vial	2 mL×1 vial	-20℃, 12 months shading light
Reagent 4	Stop Solution	12 mL×1 vial	60 mL×1 vial	-20℃, 12 month
	Microplate	96 wells	/	No requirement
	Plate Sealer	2 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:

Micropipettor, Microplate reader (400-415 nm, optimum wavelength: 405 nm), Incubator, Water bath

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate other reagents to room temperature before use.

② The preparation of substrate working solution:

Dissolve one vial of substrate with 3 mL of buffer solution. Store at -20℃ for 24 hours protected from light.

③ The preparation of 500 μmol/L standard:

Dilute 35 μL of 10 mmol/L p-Nitrophenol standard solution with 665 μL of buffer solution. Mix well to dissolve. The 500 μmol/L standard should be prepared on spot.

④ The preparation of standard curve:

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

Dilute 500 μmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 500, 400, 320, 240, 160, 80, 40, 0 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**40**	**80**	**160**	**240**	**320**	**400**	**500**
500 μmol/L standard (μL)	0	16	32	64	96	128	160	200
Buffer solution (μL)	200	184	168	136	104	72	40	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 normal saline (0.9% NaCl).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min 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) sample:

① Harvest the number of cells needed for each assay (initial recommendation 3×10^6 cells).

② Wash cells with normal saline (0.9% NaCl).

③ Homogenize 3×10^6 cells in 200-400 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min 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	10-20
Mouse serum	20-30
Rat serum	10-20
Mouse plasma	10-20
HepG2 supernatant	8-12
10% Rat kidney tissue homogenate	400-1200
10% Mouse liver tissue homogenate	10-15
10% Rat lung tissue homogenate	50-100
10% Mouse brain tissue homogenate	30-50
Human urine	1

Note: The diluent is buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

Substrate working solution and standard should be stored with shading light.

Operating steps

① Standard well: add 50 μL of standards with different concentrations into the standard wells.

Sample well: add 50 μL of sample into the sample wells.

Control well: add 50 μL of buffer solution into the control wells.

② Add 50 μL of substrate working solution to the control wells and sample wells. Add 50 μL of buffer solution to the standard wells.

③ Incubate at 37℃ for 10 min.

④ Add 100 μL of stop solution to each well.

⑤ Mix fully for 5 s with microplate reader. Measure the OD values of each well at 405 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 μmol p-nitrophenol produced by 1 L serum (plasma) per minute catalyze the substrate at 37℃ is defined as 1 activity unit.

$$\underset{(U/L)}{\text{ALP activity}} = (\Delta A\text{-}b) \div a \div T \times f$$

2. Tissue sample:

Definition: The amount of 1 μmol p-nitrophenol produced by 1 g tissue protein per minute catalyze the substrate at 37℃ is defined as 1 activity unit.

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

3. Cell sample:

Definition: The amount of 1 μmol p-nitrophenol produced by 1 g cell protein per minute catalyze the substrate at 37℃ is defined as 1 activity unit.

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

[Note]

ΔA_{530}: Absoluted OD value, $OD_{Sample} - OD_{Control}$

f: Dilution factor of sample before test.

T: Reaction time, 10 min.

C_{pr}: 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 (U/L)	0.80	12.00	35.00
%CV	1.5	1.0	1.1

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 (U/L)	0.80	12.00	35.00
%CV	4.3	4.8	4.7

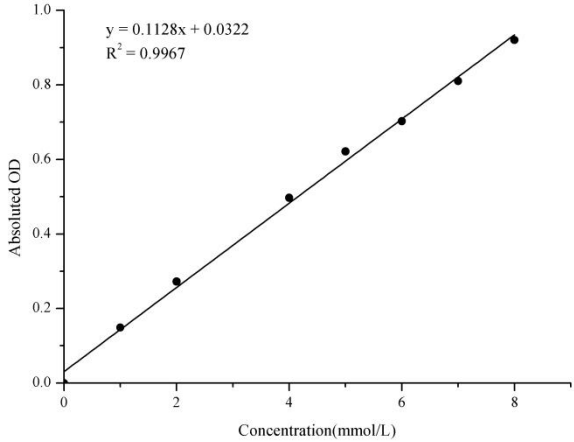
Sensitivity

The analytical sensitivity of the assay is 0.27 U/L ALP. 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 (μmol/L)	**0**	**40**	**80**	**160**	**240**	**320**	**400**	**500**
Average OD	0.050	0.159	0.267	0.474	0.684	0.902	1.095	1.374
Absoluted OD	0.000	0.110	0.217	0.424	0.634	0.852	1.045	1.324



Appendix II Example Analysis

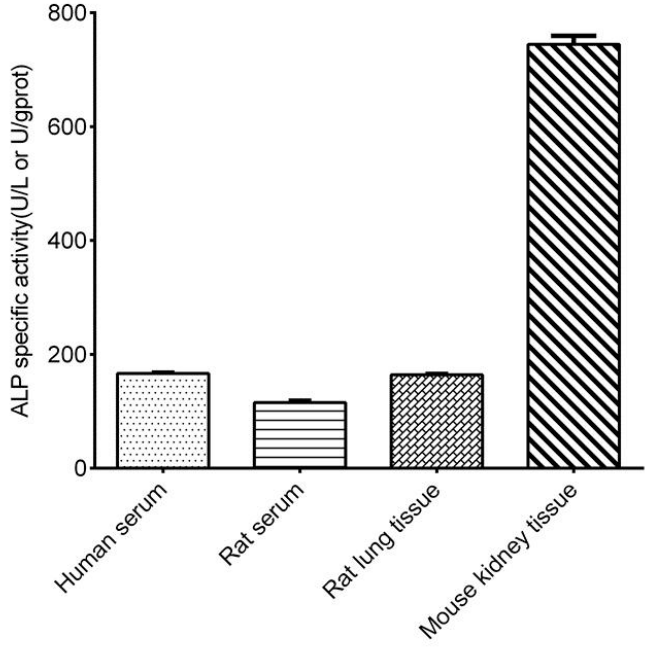
Example analysis：

For human serum, dilute human serum with buffer solution for 10 times, take 50 μL of diluted samples and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0027 x - 0.0051, the average OD value of the control is 0.099, the average OD value of the sample is 0.544, and the calculation result is:

ALP activity (U/L) = (0.544 - 0.099 + 0.0051) ÷ 0.0027 ÷ 10 × 10 = 166.70 U/L

Detect human serum (dilute for 10 times), rat serum (dilute for 10 times), 10% rat lung tissue homogenate (the concentration of protein is 3.18 gprot/L dilute for 80 times) and 10% mouse kidney tissue homogenate (the concentration of protein is 6.33 gprot/L dilute for 400 times) according to the protocol, the result is as follows：



Appendix III Publications

1. Liu Y, Wang Z, Wang Y, et al. Ca-DEX biomineralization-inducing nuts reverse oxidative stress and bone loss in rheumatoid arthritis[J]. Nanoscale, 2023, 15(33): 13822-13833.
2. 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.
3. 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.
4. Zhou Z , Jin Z , Tian Y ,et al.CDK14 is regulated by IGF2BP2 and involved in osteogenic differentiation via Wnt/ β -catenin signaling pathway in vitro[J].Life sciences, 2024, 358:123148.DOI:10.1016/j.lfs.2024.123148.
5. Zhang Y , Niu Y , Fu C ,et al.Preparation, identification and screening of anti-osteoporosis milk-derived peptides: Intervention effects in osteoporosis rats[J].Food Bioscience, 2024, 62.DOI:10.1016/j.fbio.2024.105120.

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.