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

Catalog No：E-BC-K010-M

Specification：96T(40 samples)/96T×5(284 samples)

Measuring instrument：Microplate reader（400-415 nm）

Detection range：0.2—50 U/L

Elabscience® Acid Phosphatase (ACP) 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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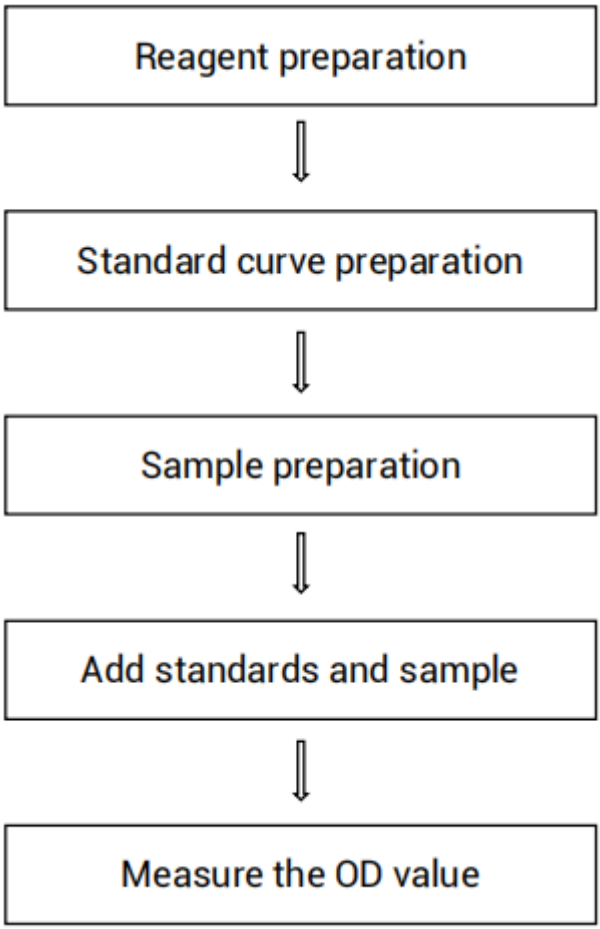
Assay summary



Intended use

This kit can be used to measure acid phosphatase (ACP) activity in serum (plasma), tissue samples.

Detection principle

Disodium p-nitrobenzene phosphate (p-NPP), a widely used phosphatase chromogenic substrate, can form p- nitrophenol under the action of acid phosphatase. Under alkaline conditions, p-nitrophenol is yellow and has a maximum absorption peak at 405 nm. The darker of the yellow product is, the higher of the acid phosphatase (ACP) activity is. Therefore, the activity of ACP can be calculated by measuring the OD value at 405 nm.

Kit components & storage

Item	Component	Size 1 (96 T)	Size 2(96T×5)	Storage
Reagent 1	Buffer Solution	20 mL×1 vial	50 mL×2 vials	-20℃, 12 months
Reagent 2	Substrate	Powder×3 vials	Powder×15 vials	-20℃, 12 months shading light
Reagent 3	Standard	Powder×1 vial	Powder×5 vials	-20℃, 12 months shading light
Reagent 4	Chromogenic Agent	24 mL×1 vial	60 mL×2 vials	-20℃, 12 months
	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:

Micropipettor, Microplate reader (400-415 nm, optimum wavelength: 405 nm), 37℃ Incubator

Reagents:

Double distilled water, PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of substrate working solution：
Dissolve one vial of substrate with 1.6 mL of buffer solution. Store at -20°C for 24 hours protected from light.

③ The preparation of 10 mmol/L standard stocking solution：
Dissolve one vial of standard with 5 mL of double distilled water, mix well. Aliquoted storage at -20°C for 7 days protected from light.

④ The preparation of 0.5 mmol/L standard：
Dilute 36 μL of standard stocking solution with 684 μL of buffer solution. Mix well to dissolve. The 0.5 mmol/L standard should be prepared on spot.

⑤ The preparation of standard curve：

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

Dilute 0.5 mmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.05, 0.1, 0.2, 0.25, 0.3, 0.4, 0.5 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.05**	**0.1**	**0.2**	**0.25**	**0.3**	**0.4**	**0.5**
0.5 mmol/L standard (μL)	0	20	40	80	100	120	160	200
Buffer solution (μL)	200	180	160	120	100	80	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 PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL PBS (0.01 M, pH 7.4) 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).

② **Dilution of sample**

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

Sample type	Dilution factor
10% Epipremnum aureum tissue homogenate	5-10
Mouse plasma	5-10
Rat plasma	5-10
Human urine	1
Human plasma	5-10
10% Rat spleen tissue homogenate	20-30
10% Rat liver tissue homogenate	20-30
10% Rat kidney tissue homogenate	20-30

Note: The diluent is 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

① Substrate working solution and standard should be stored with shading light.

② Substrate working solution should be used up within 1 day.

Operating steps

① Standard well: add 40 μL of standards with different concentrations into the standard wells.
Sample well: add 40 μL of sample into the sample wells.
Control well: add 40 μL of sample into the control wells.

② Add 40 μL of buffer solution to the standard wells and control wells.

③ Add 40 μL of substrate working solution to the sample wells.

④ Mix fully for 3 s with microplate reader and incubate at 37℃ for 10 min.

⑤ Add 160 μL of chromogenic agent to each well.

⑥ Mix fully for 3 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 hydrolysis PNPP at 37℃ is defined as 1 activity unit.

$$\text{ACP activity (U/L)} = (\Delta A - b) \div a \div T \times f \times 1000*$$

2. Tissue sample:

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

$$\text{ACP activity (U/L)} = (\Delta A - b) \div a \div T \times f \times 1000* \div C_{pr}$$

[Note]

ΔA_{405}: 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.

1000*: 1 mmol=1000 μmoL.

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)	0.50	15.00	38.00
%CV	3.2	2.6	2.6

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)	0.50	15.00	38.00
%CV	4.3	4.7	4.5

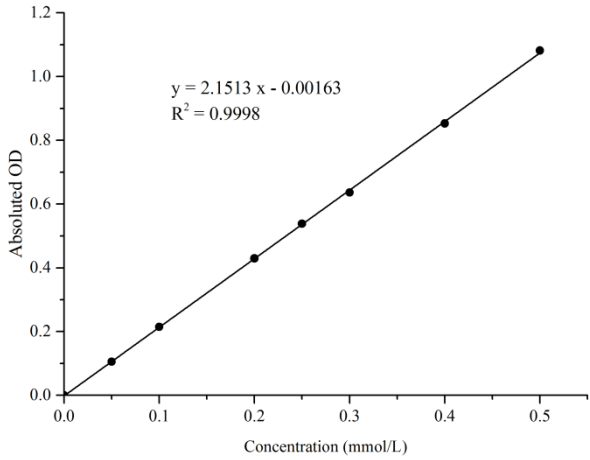
Sensitivity

The analytical sensitivity of the assay is 0.2 U/L ACP. 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 (mmol/L)	**0**	**0.05**	**0.1**	**0.2**	**0.25**	**0.3**	**0.4**	**0.5**
Average OD	0.039	0.145	0.469	0.469	0.577	0.675	0.892	1.121
Absoluted OD	0	0.105	0.214	0.429	0.538	0.626	0.852	1.081



Appendix Π Example Analysis

Example analysis：

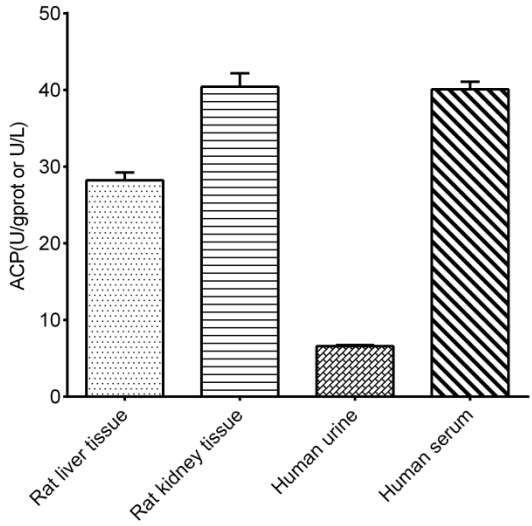
For rat kidney tissue, take 10% rat kidney tissue homogenate diluted for 20 times and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 2.1298 x+0.0025, the average OD value of the sample is 0.470, the average OD value of the control is 0.06, the concentration of protein in sample is 9.47 gprot/L, and the calculation result is:

ACP activity (U/gprot) = (0.470 - 0.06 - 0.0025 ÷ 2.1298 ÷ 10 ÷ 9.47 × 20 × 1000

= 40.4 U/gprot

Detect 10% rat liver tissue homogenate (the concentration of protein is 12.22 gprot/L dilute for 20 times), 10% rat kidney tissue homogenate (the concentration of protein is 9.47 gprot/L dilute for 20 times), human urine and human serum (dilute for 10 times) 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.