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

Catalog No：E-BC-K094-M

Specification：96T(80 samples)/500Assays(484 samples)

Measuring instrument：Microplate reader（510-530 nm）

Detection range：1.40-40 U/100 mL

Elabscience®Acid Phosphatase (ACP) 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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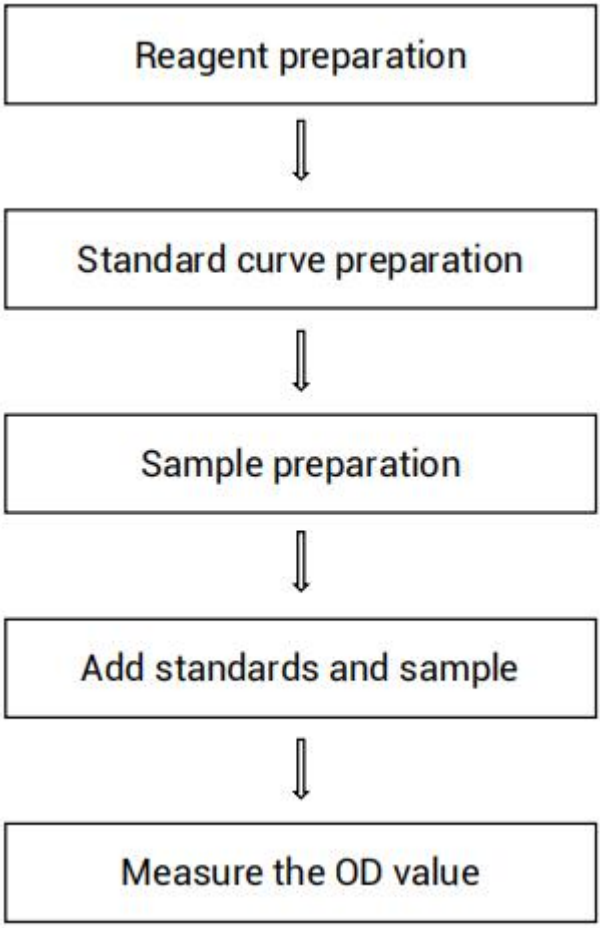
Assay summary

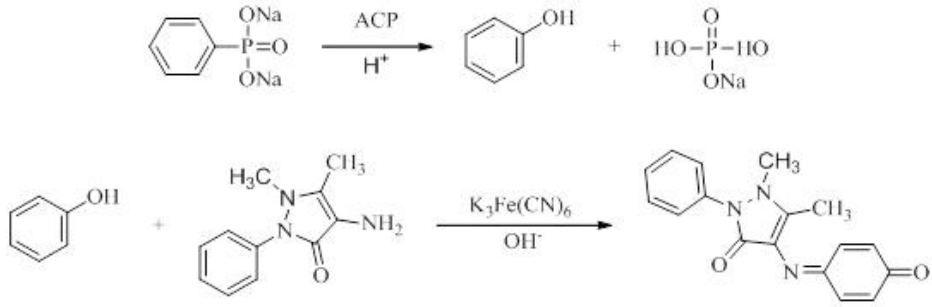


Intended use

This kit can be used to measure acid phosphatase (ACP) activity in serum (plasma), hydrothorax, urine, cells and cell culture supernatant and animal tissue samples.

Detection principle

Acid phosphatase decomposes disodium phenyl phosphate under acidic conditions to produce free phenol and phosphoric acid. Phenol acts with 4-aminoantipyrine in alkaline solution, and oxidizes to a derivative of red quinone by potassium ferricyanide. The activity of the ACP can be calculated by measuring the OD value at 520 nm.



Kit components & storage

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 1	Buffer Solution	2 mL × 1 vial	10 mL × 1 vial	2-8℃, 12 months
Reagent 2	Substrate Solution	2 mL × 1 vial	10 mL × 1 vial	2-8℃, 12 month shading light
Reagent 3	Alkali Reagent	6 mL × 2 vials	60 mL × 1 vial	2-8℃, 12 month shading light
Reagent 4	Chromogenic Agent	9 mL × 2 vials	45 mL × 2 vials	2-8℃, 12 month shading light
Reagent 5	1.0 mg/mL Phenol Standard	2 mL × 1 vial	10 mL × 1 vial	2-8℃, 12 month shading light
	Microplate	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 (510-530 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，add 12.5 μL of buffer solution and 12.5 μL of substrate solution, mix well. The working solution should be prepared on spot. Store at 2-8°C for 1 day.

③ The preparation of standard curve：

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

Dilute 1 mg/mL phenol standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.4, 0.5, 0.6, 0.8, 1 mg/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mg/mL)	**0**	**0.1**	**0.2**	**0.4**	**0.5**	**0.6**	**0.8**	**1.0**
1 mg/mL phenol standard (μL)	0	20	40	80	100	120	160	200
Double distilled water (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 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 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) 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 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 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	1
Humasn saliva	1
Chicken serum	1
Human urine	1
10% Rat liver tissue homogenate	4-10
10% Rat kidney tissue homogenate	4-10
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 25 μL of working solution and mix fully for 10 s with microplate reader.

③ Incubate at 37℃ for 30 min, then add 100 μL of alkali reagent and 150 μL of chromogenic agent, mix fully for 10 s with microplate reader.

④ Stand for 5 min at room temperature and 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 30 min is defined as 1 unit.

$$\text{ACP activity (U/100 mL)} = (\Delta A - b) \div a \times V \times f$$

2. Tissue and cells sample:

Definition: The amount of 1 mg phenol produced by 1 g tissue protein react with the substrate in 30 min is defined as 1 unit.

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

[Note]

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

V: 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 (U/100 mL)	3.70	26.80	32.50
%CV	2.5	2.0	1.8

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/100 mL)	3.70	26.80	32.50
%CV	9.4	9.2	9.6

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 104%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mg/mL)	0.15	0.45	0.77
Observed Conc. (mg/mL)	0.2	0.5	0.8
recovery rate(%)	101	106	105

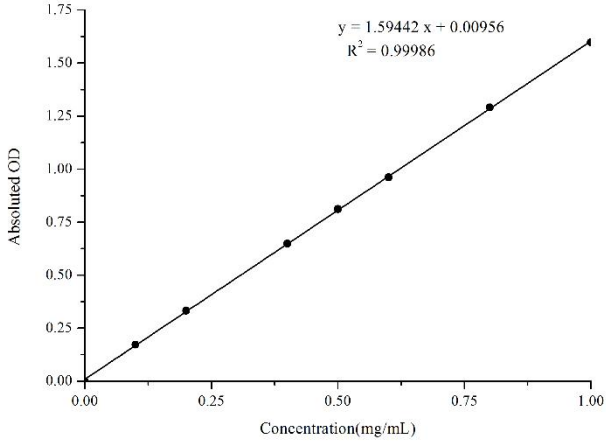
Sensitivity

The analytical sensitivity of the assay is 1.40 U/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**	**0.1**	**0.2**	**0.4**	**0.5**	**0.6**	**0.8**	**1**
Average OD	0.061	0.234	0.394	0.710	0.873	1.022	1.352	1.659
Absoluted OD	0	0.173	0.333	0.649	0.812	0.961	1.291	1.598



Appendix Π Example Analysis

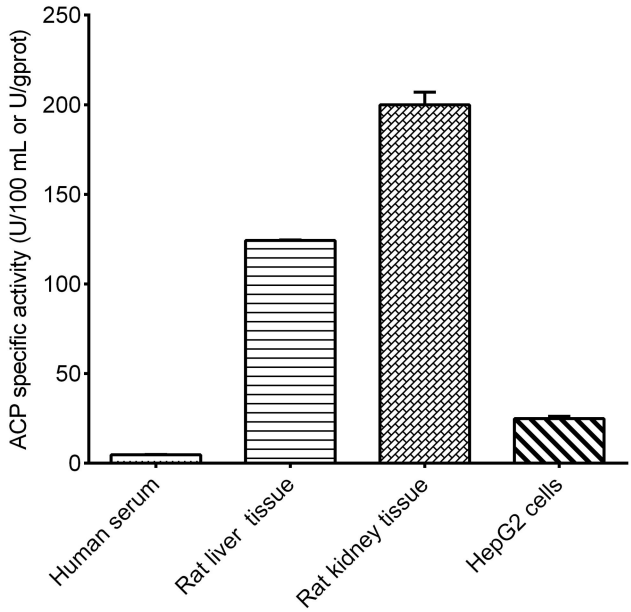
Example analysis：

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

Standard curve: y = 1.59442 x + 0.00956, the average OD value of the sample is 0.153, the average OD value of the blank is 0.071, and the calculation result is:

ACP (U/100 mL) = (0.082 - 0.00956) ÷ 1.59442 × 100 = 4.54 U/100 mL

Detect human serum, 10% rat liver tissue homogenate (the concentration of protein is 0.013 gprot/mL, dilute for 10 times), 10% rat kidney tissue homogenate (the concentration of protein is 0.007 gprot/mL, dilute for 10 times) and HepG2 cells (the concentration of protein is 0.010 gprot/mL) 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.