Acid Phosphatase (ACP) Activity Assay Kit

Catalog No: E-BC-K094-S

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.27 U/100 mL

Detection range: 0.27-40 U/100 mL

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲Background

Acid phosphatase (ACP) is a kind of enzyme which catalyzes the hydrolysis of phosphate monoester to phosphoric acid under acidic conditions. There are different acid phosphatase isozymes in different organs. These isozymes differ greatly in tissue and chromosome origin, molecular weight, amino acid homology, sequence length, and resistance to L-tartrate or fluoride.

▲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	Specification	Storage
Reagent 1	Buffer Solution	60 mL × 1 vial	2-8℃ , 3 months
Reagent 2	Substrate Solution	60 mL × 1 vial	2-8℃ , 3 months, shading light
Reagent 3	Alkali Reagent	60 mL × 2 vials	2-8℃ , 3 months, shading light
Reagent 4	Chromogenic Agent	60 mL × 3 vials	2-8℃ , 3 months, shading light
Reagent 5	1 mg/mL Phenol Standard	1 mL ×1 vial	2-8℃ , 3 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.			

Materials prepared by users

Instruments

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

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 mL)

Reagents

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

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key points of the assay

Add reagent 3 and reagent 4 immediately after incubating at 37 ℃ for 30 min.

Pre-assay preparation

▲Reagent preparation

Preparation of 0.1 mg/mL phenol standard application solution

Dilute reagent 5 with double distilled water at a ratio of 1:9 and mix fully. Prepared the fresh solution before use.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

Sample requirements

Samples should not contain oxalate or fluoride.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.27-40 U/100 mL).

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

Sample type	Dilution factor
Human milk	1
Human saliva	1
Human urine	1
Human serum	1
HepG2 cells homogenization	2-8
10% Mouse kidney tissue homogenization	8-12
10% Mouse liver tissue homogenization	8-12
10% Mouse spleen tissue homogenization	8-12

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	520 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operating steps

1. Blank tube: add 50 μL of double distilled water into a 5 mL EP tube.
   Standard tube: add 50 μL of 0.1 mg/mL phenol standard application solution into a 5 mL EP tube.
   Sample tube: add 50 μL of sample into a 5 mL EP tube.
2. Successively add 500 μL of reagent 1 and 500 μL of reagent 2 respectively and oscillate fully with the vortex mixer.
3. Incubate at 37℃ for 30 min, then add 1000 μL of reagent 3 and 1500 μL of reagent 4 immediately, oscillate fully with the vortex mixer and stand at room temperature for 10 min.
4. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 520 nm with 1 cm optical path quartz cuvette.

Operation table

	Blank tube	Standard tube	Sample tube
Double distilled water (μL)	50		
0.1 mg/mL Phenol standard application solution (μL)		50	
Sample (μL)			50
Reagent 1 (μL)	500	500	500
Reagent 2 (μL)	500	500	500
Mix fully and incubate at 37℃ for 30 min.			
Reagent 3 (μL)	1000	1000	1000
Reagent 4 (μL)	1500	1500	1500
Mix fully immediately and stand at room temperature for 10 min, then set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 520 nm with 1 cm optical path quartz cuvette.			

Calculation

For serum (plasma) and other liquid samples

(1) Definition

100 mL of sample reacts with the substrate at 37℃ for 30 min to produce 1 mg of phenol that is defined as 1 unit.

(2) Calculation formula

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

For tissue or cell samples

(1) Definition

1 g of tissue protein reacts with the substrate at 37℃ for 30 min to produce 1 mg of phenol that is defined as 1 unit.

(2) Calculation formula

$$\text{ACP activity (U/gprot)} = \frac{\Delta A_1}{\Delta A_2} \times m \div [C_{pr} \times V] \times f$$

Note:

ΔA_1: $OD_{Sample} - OD_{Blank}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

m: Phenol content of standard tube, 0.005 mg

Cpr: Protein concentration of tested sample, gprot/mL

V: The volume of sample, 0.05 mL

V1: The volume of sample in definition, 100 mL

f: Dilution factor of sample before test.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 3 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	0.27-40 U/100 mL	Average intra-assay CV (%)	2.8
Sensitivity	0.27 U/100 mL	Average inter-assay CV (%)	7.1
Average recovery rate (%)	100		

▲Example analysis

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

The average OD value of the sample is 0.200, the average OD value of the blank is 0.037, the average OD value of the standard is 0.215, and the calculation result is:

$$\text{ACP activity (U/100 mL)} = (0.200-0.037) \div (0.215-0.037)\times0.005\times(100\div0.05)$$

$$=9.16 \text{ (U/100 mL)}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃. Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲ Plasma

Take fresh blood into the tube which has anticoagulant (heparin is recommended), centrifuge at 700-1000 g for 10 min at 4℃. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

▲ Urine

Collect fresh urine and centrifuge at 10000 g for 15 min at 4℃. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine can be stored at -80℃ for a month.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4℃. Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S). If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

▲ Cells

Collect the cells (For adherent cells, the cell scraper rather than trypsin is recommended.) and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^6): homogenization medium (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S). If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ for a month.

Note:

1. Homogenized medium: PBS (0.01 M, pH 7.4) or 0.9% NaCl.
2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 cm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/ time, interval for 3 s, the total time is 5 min).

▲ Notes for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
3. If a lysis buffer is used to prepare tissue or cell homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.