Acid Phosphatase (ACP) Activity Assay Kit

Catalog No: E-BC-K094-M

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 1.40 U/100 mL

Detection range: 1.40-40 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

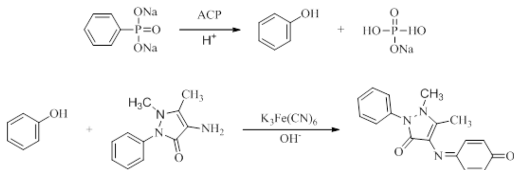
This kit can be used to measure acid phosphatase (ACP) activity in serum (plasma), hydrothorax, urine, cells and cell culture supernatant and animal tissue samples.

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	2 mL × 1 vial	2-8°C , 3 months
Reagent 2	Substrate Solution	2 mL × 1 vial	2-8°C , 3 months, shading light
Reagent 3	Alkali Reagent	6 mL × 2 vials	2-8°C , 3 months, shading light
Reagent 4	Chromogenic Agent	9 mL × 2 vials	2-8°C , 3 months, shading light
Reagent 5	1 mg/mL Phenol Standard	2 mL × 1 vial	2-8°C , 3 months, shading light
	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.

Materials prepared by users

Instruments

Microplate reader (510-530 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Consumptive material

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

Pre-assay preparation

▲Reagent preparation

The preparation of working solution

Mix the reagent 1 and reagent 2 at the ratio of 1:1 fully. Prepare the fresh solution before use and the unused solution can be stored at 2-8°C with shading light for 24 hours.

▲Sample preparation

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

Sample requirements

The sample should not contain oxalate and 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 (1.40-40 U/100 mL).

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
HepG2 cells	1

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

Assay protocol	
Ambient temperature	25-30°C
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

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

▲Operating steps

The preparation of standard curve

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.

The measurement of samples

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°C for 30 min, then add 100 μL of reagent 3 and 150 μL of reagent 4, 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.

▲Operation table

	Standard well	Sample well
Standards with different concentrations (μL)	5	
Sample (μL)		5
Working solution (μL)	25	25
Mix fully for 10 s with microplate reader and incubate at 37°C for 30 min.		
Reagent 3 (μL)	100	100
Reagent 4 (μL)	150	150
Mix fully for 10 s with microplate reader, stand for 5 min at room temperature and measure the OD values of each well at 520 nm with microplate reader.		

Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample.
The standard curve is: y= ax + b.

1. Serum (plasma) and other liquid 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.

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

2. Tissue and cells

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.

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

Note:

y: The absolute OD value of standard;

x: The concentration of Standard;

a: The slope of standard curve;

b: The intercept of standard curve.

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

▲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	1.40-40 U/100 mL	Average intra-assay CV (%)	2.1
Sensitivity	1.40 U/100 mL	Average inter-assay CV (%)	9.4
Average recovery rate (%)	104		

▲Example analysis

Take 5 μL of human serum and carry the assay according to the operation table. 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, the absolute OD is 0.082, and the calculation result is:

$$\text{ACP (U/100 mL)} = \frac{0.082 - 0.00956}{1.59442} \times 100 = 4.54 \text{ (U/100 mL)}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C . 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°C 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°C . 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°C for a month.

▲ Urine

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

▲ Hydrothorax

Collect the fresh hydrothorax to the tubes with anticoagulant (heparin is recommended) and mix fully. Centrifuge the sample at 10000 g for 10 min at 4°C , then take the supernatant and preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80°C 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). If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

▲ Cells

Collect the cells (do not use trypsin and EDTA) 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). If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ for a month.

Note:

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

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^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).

▲ Note 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.