Acid Phosphatase (ACP) Activity Assay Kit

Catalog No: E-BC-K010-M

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.2 U/L

Detection range: 0.2–50 U/L

- 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), tissue samples.

▲Background

Acid phosphatase (ACP) is a kind of acidic hydrolytic enzyme with high content in lysosomes which catalyzes the hydrolysis of phosphate monoester to phosphoric acid under acidic conditions. ACP is ubiquitous in nature, from low organisms such as escherichia coli and yeast to higher animal and plant tissues, as well as body fluids, human liver, and prostate.

▲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 ACP activity is. Therefore, the activity of ACP can be calculated by measuring the OD value at 405 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	20 mL×1 vial	-20℃ , 3 months
Reagent 2	Substrate	Powder ×3 vials	-20℃ , 3 months, shading light
Reagent 3	5 mmol/L Standard	0.8 mL × 1 vial	-20℃ , 3 months, shading light
Reagent 4	Stop Solution	24 mL × 1 vial	-20℃ , 3 months
	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

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

Reagents

Double distilled water, 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

1. Reagent 2 working solution and standard should be stored with shading light.
2. Reagent 2 working solution should be used up within 1 day.

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents to room temperature before use.
2. The preparation of reagent 2 working solution:

   Dissolve reagent 2 with 1.6 mL of reagent 1. The prepared solution can be stored at -20°C with shading light for 24 hours.

3. The preparation of 0.5 mmol/L standard:

   Mix the reagent 3 and reagent 1 at the ratio of 1:9 fully. Prepare the fresh solution before use and store with shading light for detection.

▲Sample preparation

1. Serum (Plasma): Detect the sample directly.
2. Tissue sample: Weigh the tissue accurately. Add PBS (0.01 M，pH 7.4) in a weight (g): volume (mL) ratio of 1: 9, homogenize mechanically in ice water bath to break tissue fully. Then centrifuge at 10000 g for 10 min at 4°C and collect the supernatant for measurement. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

▲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 (0.2–50 U/L).

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).

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	405 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	S1'	S9'	S17'	S25'	S33'
B	B	B	S2	S10	S18	S26	S34	S2'	S10'	S18'	S26'	S34'
C	C	C	S3	S11	S19	S27	S35	S3'	S11'	S19'	S27'	S35'
D	D	D	S4	S12	S20	S28	S36	S4'	S12'	S20'	S28'	S36'
E	E	E	S5	S13	S21	S29	S37	S5'	S13'	S21'	S29'	S37'
F	F	F	S6	S14	S22	S30	S38	S6'	S14'	S22'	S30'	S38'
G	G	G	S7	S15	S23	S31	S39	S7'	S15'	S23'	S31'	S39'
H	H	H	S8	S16	S24	S32	S40	S8'	S16'	S24'	S32'	S40'

Note: A-H, standard wells; S1-S40, sample wells; S1′-S40′, control wells.

▲Operating steps

The preparation of standard curve

Dilute 0.5 mmol/L standard solution with reagent 1 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.

The measurement of samples

1. Standard well: Take 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 reagent 1 into the standard wells and control wells.
   Add 40 μL of reagent 2 working solution into the sample wells
3. Mix fully with microplate reader for 3 s and incubate at 37°C for 10 min
4. Add 160 μL of reagent 4 into each well.
5. Mix fully with microplate reader for 3 s. Measure the OD values of each well at 405 nm with microplate reader.

▲Operation table

	Standard well	Sample well	Control well
Standards with different concentrations (μL)	40		
Sample (μL)		40	40
Reagent 1 (μL)	40		40
Reagent 2 Working solution (μL)		40	
Mix fully with microplate reader for 3 s and incubate at 37°C for 10 min.			
Reagent 4 (μL)	160	160	160
Mix fully with microplate reader for 3 s. Measure the OD values of each well at 405 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) sample

Definition: The amount of 1 μmol p-nitrophenol produced by 1 L serum (plasma) per minute hydrolysis PNPP at 37°C 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°C 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:

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0);
x: The concentration of Standard;
a: The slope of standard curve;
b: The intercept of standard curve;
ΔA: ($OD_{Sample} - OD_{Control}$);
f: Dilution factor of sample before test;
T: Reaction time, 10 min;
C_{pr}: Concentration of protein in tissue sample, gprot/L;
1000*: 1 mmol=1000 μmoL

▲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	0.2–50 U/L	Average intra-assay CV (%)	2.8
Sensitivity	0.2 U/L	Average inter-assay CV (%)	4.5

▲Example analysis

For rat kidney tissue, take10% rat kidney tissue homogenate diluted for 20 times and carry the assay according to the operation table. 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