Acid Phosphatase (ACP) Test Kit

(Cat.No:BC103 Size:96T, 48T)

1. Principle of Measurement

The acidic phosphatase decomposes sodium phytate, generating free phenols and phosphate. Phenol reacts with 4-aminophenylacridine in an alkaline solution and is oxidized by potassium ferrocyanide to form a red quinone derivative. The intensity of the red color can be used to determine the level of enzyme activity.

2. Composition and Preparation (The kit is valid for 6 months)

	Component	48T	96T	Preservation Condition
Reagent I	Buffer	3mL×1 bottle	5mL×1 bottle	4℃
Reagent II	Matrix Solution	3mL×1 bottle	5mL×1 bottle	-20℃
Reagent III	Alkaline Solution	5mL×1 bottle	10mL×1 bottle	4℃ in the dark
Reagent IV	Color developer	5mL×1 bottle	10mL×1 bottle	4℃ in the dark
Reagent V	1.1mg/mL Stocking Solution	0.5mL×1 vial	0.5mL×1 vial	4℃ in the dark
0.1mg/mL Phenol standard solution preparation: Prepare the phenol standard stock solution at 1.1 mg/mL: Dilute with distilled water in a ratio of 1:10. Prepare it as needed.				

3. Sample collection and preservation

1. According to the conventional method, samples can be collected from serum, plasma (with heparin anticoagulation being preferred), cell culture supernatant, tissues or cultured cells.
2. After sample collection (such as serum (plasma), tissue, cultured cells, culture supernatant, etc.), if timely detection is not possible, please store the samples at temperatures below -20℃ (the lower the temperature, the better).

4. Required instruments and reagents

An adjustable enzyme detector with a wavelength range of 490-530 nm and a 96-well plate (included), a 37℃ constant temperature box or water bath, distilled water, physiological saline, protein determination reagent (for tissue or cell samples, available from our company).

5. Sample pre-treatment

1. **Serum (plasma):** After thawing, it can be used directly (for some samples with excessively high content, they need to be diluted before measurement; for example, chicken serum should be diluted 5 to 10 times with physiological saline).
2. **Cell culture medium:** Siphon out the culture medium and add it to the centrifuge tube. Centrifuge at 2000 - 4000 rpm for 5 minutes, then take the supernatant for testing.

3.Sample of the organization: Accurately weigh the sample to be tested. Add 9 times the volume of normal saline (in milliliters) according to the ratio of weight (grams) : volume (milliliters) = 1 : 9. Perform mechanical homogenization under an ice water bath at 2500 revolutions per minute for 10 minutes. Take the supernatant of the homogenate for testing. (The supernatant of the homogenate needs to be determined for its protein concentration. This reagent kit is available from our company: item number A045-2 or A045-3/-4)

4. Cell sample: Firstly, for **suspended cultured cells**, the precipitated cells can be directly collected by centrifugation (at 1000 revolutions per minute for 10 minutes, discarding the supernatant and retaining the precipitated cells). For **adherent cells**, first aspirate the culture medium. The cells can then be harvested either by direct scraping with a cell scraper, or by digestion with 0.25% trypsin at room temperature for 2–3 minutes. Add fresh culture medium to terminate the digestion, gently pipette the mixture up and down, aspirate all the liquid and transfer it to an EP tube. Centrifuge the tube at 1,000 rpm for 10 minutes, discard the supernatant and retain the cell pellet. Resuspend the pellet gently in 1 mL of PBS by pipetting, then centrifuge again under the same conditions (1,000 rpm for 10 minutes). Discard the supernatant and keep the cell pellet for subsequent use.(If the experiment is not to be performed immediately, the cell pellet can be cryopreserved at low temperature; the lower the temperature, the better.)Add an appropriate volume of buffer (0.3–0.5 mL for 1×10^6 cells; buffer can be PBS or physiological saline) to the collected cell pellet, followed by cell lysis. **The first crushing method** is to use a manual glass homogenizer and conduct the grinding in an ice-water bath for 3 to 5 minutes, or to use an electric homogenizer and perform the grinding for 3 minutes before testing. **The second crushing method** is ultrasonic disruption (make sure the ultrasonic probe is below the liquid surface, with a power of 300W, in an ice-water bath. Perform the ultrasonic disruption once every 3 to 5 seconds, with a 4-second interval (each interval lasts about 30 seconds).) **The third crushing method** is the chemical lysis method (for adherent cultured cells, the supernatant can be directly removed and a certain amount of lysis solution (enough to cover the cells) can be added directly to the well plate or bottle. Lysis should be carried out for 30 to 40 minutes (cell lysis can be observed under a microscope), and then the supernatant can be aspirated out for testing.Depending on the requirements, normal saline or PBS can be used for a certain degree of dilution; after the cells are disrupted, centrifuge at 2500 revolutions per minute for 10 minutes, and then take the supernatant for testing.

6. Operation Table

	Blank	Standard	Sample
DDW (μL)	4		
0.1 mg/mL phenol standard solution (μL)		4	
Sample (μL)			4
Buffer solution (μL)	40	40	40
Matrix fluid (μL)	40	40	40
Mix thoroughly and warm at 37°C for 30 min			
Alkali solution (μL)	80	80	80
Color developer (μL)	80	80	80
Gently shake the plate to mix evenly, let it stand for 10 minutes, with			

a wavelength of 520 nm, and measure the absorbance of each well using an enzyme detector.

7. Technical Specifications

1	Blank well	≤0.150
2	Kit Intra-batch CV	≤3%
3	Kit Inter-batch CV	≤5%
4	Reagent kit recovery rate	98%
5	Linear range: 0 - 60 Jin units/100 mL	R^2=0.999
6	Wavelength selection range	490nm ~ 530nm

8. Calculation formula

1. Liquid Sample Calculation Formula: (Applicable to culture medium, serum, plasma, and other liquid samples)

Definition: 100 mL of serum or liquid reacts with the matrix at 37℃ for 30 minutes, resulting in 1 mg of phenol being equivalent to 1 King unit.

Calculation Formula:

$$\textbf{ACP Activity in Liquid Samples}=\frac{A_{measured}-A_{blank}}{A_{standard}-A_{blank}} \times C_{standard} \times 100 \times N$$

$\boldsymbol{C_{standard}}$**:** Phenol standard concentration: 0.1 mg/mL;
N: Sample Pre-test Dilution Factor

For example:

Example 1: Take 4 μL of the crab monkey serum and perform the ACP determination according to the operation table. The absorbance of the blank well is 0.0436, the absorbance of the standard well is 0.1845, and the absorbance of the test well is 0.1324. Then, the calculation is as follows:

ACP Activity in Cynomolgus Monkey Serum (King unit/100mL)=

$$\frac{0.1324-0.0436}{0.1845-0.0436} \times 0.1 \times 100 = 6.3023 \text{ King unit}/100\text{mL}$$

Example 2: Take 4 μL of mouse serum and perform the ACP determination according to the operation table. The absorbance of the blank well is 0.0436, the absorbance of the standard well is 0.1845, and the absorbance of the test well is 0.2575. Then, the calculation is as follows:

ACP Activity in Mouse Serum (King unit/100mL)=

$$\frac{0.2575-0.0436}{0.1845-0.0436} \times 0.1 \times 100 = 15.1809 \ King\ unit/100mL$$

2. Tissue Calculation Formula: (Applicable to cultured cells, tissues, and other related samples)

Definition: Each gram of tissue protein, when acted upon by the matrix at 37℃ for 30 minutes, produces 1 mg of phenol, which is equivalent to 1 King unit.

Calculation Formula:

$$\textbf{ACP Activity in Tissues/Cells (King unit/g protein)}=\frac{A_{measured}-A_{blank}}{A_{standard}-A_{blank}}\times C_{standard}\div Cpr$$

Cpr: Sample protein concentration, gprot/mL (prot refers to protein)

Example 3: Take 4 μL of 10% mouse liver tissue homogenate after 1:1 dilution with physiological saline for ACP assay. The absorbance of the blank tube is 0.0436, the standard absorbance is 0.1845, and the measured absorbance is 0.6013. Meanwhile, the protein concentration of this 5% rat liver tissue homogenate was determined to be 9.8327×10^{-3} gprot/mL. The calculation is as follows:

Mouse ACP Activity (King unit/gprot)=

$$\frac{0.6013-0.0436}{0.1845-0.0436}\times 0.1\div(9.8327\times10^{-3})=40.2547\ \text{King unit}/100\text{mL}$$

Example 4: Take 4 μL of 10% mouse lung tissue homogenate for ACP assay. The absorbance of the blank tube is 0.0440, the standard absorbance is 0.2426, and the measured absorbance is 0.1392. Meanwhile, the protein concentration of this 10% mouse lung tissue homogenate is determined to be 5.1182×10^{-3} gprot/mL. The calculation is as follows:

Mouse Lung ACP Activity (King unit/gprot)=

$$\frac{0.1392-0.0440}{0.2426-0.0440}\times 0.1\div(5.1182\times10^{-3})=46.0511\ \text{King unit}/100\text{mL}$$

9. Notes

1. If the microplate reader in use does not have this wavelength, adjacent wavelengths (510 nm or 530 nm) can be used instead; the detection results at 510 nm are relatively better than those at 530 nm.
2. Since the sample volume is relatively small, it is recommended to place the pipette tip close to the bottom of the microplate during pipetting, add the sample slowly, and gradually move the tip upward while dispensing. This minimizes sample residue on the tip and reduces pipetting-related errors.
3. The reagents added are close to aqueous solutions, so they have very little adhesion to the pipette tip. However, when adding the reagents, caution is still necessary. The speed should not be too fast to prevent the reagents from spilling out of the enzyme-labeled well.
4. If adding samples or reagents along the wall, they should be added close to the bottom. The initial part of the reaction solution contains less volume. If added close to the upper end, some of it will stick to the upper part of the enzyme-labeled well, resulting in incomplete reaction.
5. Microplate wells are relatively small, so the mixing force should be moderate: excessive force may cause the liquid to splash, while insufficient force will lead to inadequate mixing. First, gently tap down the liquid adhering to the well walls, then shake the plate back-and-forth and left-and-right.
6. Generally, there may be initial absorbance differences for the enzyme-linked plates. It is best to measure the initial absorbance at the corresponding wavelength before using them, and then add the samples for measurement.

Appendix I: Preparation of ACP Standard Curve

一．Sample Pretreatment:

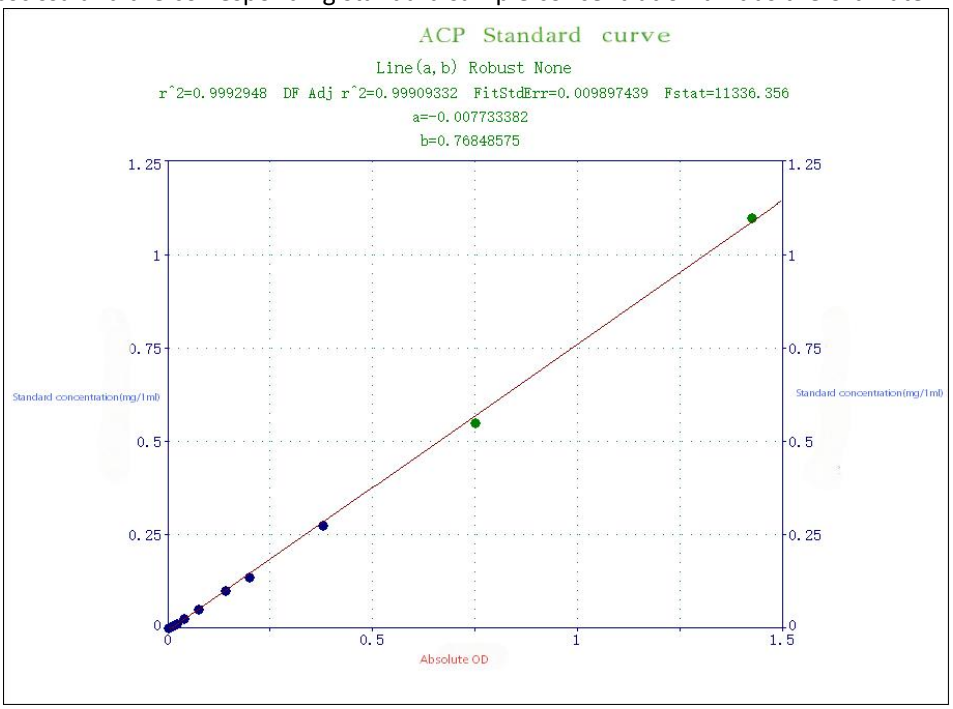
The standard stock solution was diluted with double-distilled water at ratios of 2 times, 4 times, 8 times, 11 times, 22 times, 44 times, 88 times, and 176 times. The concentrations after dilution were 0.00625 mg/mL, 0.0125 mg/mL, 0.025 mg/mL, 0.05 mg/mL, 0.1 mg/mL, 0.1375 mg/mL, 0.275 mg/mL, 0.55 mg/mL, and 1.1 mg/mL.

二．Operation Table:

	Blank	Standard
DDW (μL)	4	
Phenol Standard Solutions (Different Concentrations) (μL)		4
Buffer solution (μL)	40	40
Matrix fluid (μL)	40	40
Mix thoroughly and warm at 37°C for 30 min		
Alkali solution (μL)	80	80
Color developer (μL)	80	80
Gently shake the plate to mix evenly, let it stand for 10 minutes, with a wavelength of 520 nm, and measure the absorbance of each well using an enzyme detector.		

三．Test results:

Plot a standard curve by taking the measured absolute absorbance (OD value) as the abscissa and the corresponding standard sample concentration unit as the ordinate.



Note: The standard curve is provided for reference only. It can be omitted if desired, and calculations can be performed directly using the formula without any impact on the measurement and results.