Alkaline Phosphatase (AKP) Test Kit

(Cat/No.:BC127 Size:96T,48T)

1. Measurement Principle (Microplate Method)

Alkaline phosphatase breaks down disodium phenyl phosphate to produce free phenol and phosphoric acid. The phenol reacts with 4-aminoantipyrrolidone in an alkaline solution and is oxidized by potassium ferricyanide to produce a red quinone derivative. The intensity of the red color can be used to determine the enzyme activity.

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

	Components	48T	96T	Storage conditions
Reagent 1	buffer solution	3mL x 1 bottle	6mL x 1 bottle	Refrigerate at 4℃
Reagent 2	matrix solution	3mL x 1 bottle	6mL x 1 bottle	-20℃, away from light
Reagent 3	color developer	9mL x 1 bottle	18mL x 1 bottle	4℃, away from light
Reagent 4	1.1 mg/mL Phenol Standard Stock Solution	0.5mL × 1 vial	0.5mL × 1 vial	4℃, away from light
0.1 mg/mL phenol standard solution : 1.1 mg/mL phenol standard stock solution : distilled water = 1:10 dilution. Prepare fresh before use.				

3. Sample Collection and Preservation

1) Collect samples according to standard procedures. Samples can be serum, plasma (heparin anticoagulation is preferred), cell culture supernatant, tissue, or cultured cells (pre-treat samples according to experimental methodology before measurement).

2) If the sample cannot be tested immediately after collection (such as serum (plasma), tissue, cultured cells, culture supernatant, etc.) , please store the sample at -20℃ or below (the lower the temperature, the better).

4. Required Instruments and Reagents

Adjustable wavelength 490-530nm microplate reader and 96-well plate (one plate included), 37℃ water bath or incubator, distilled water, protein assay reagent (for animal tissues or cells, available from our company).

5. Operating Procedures

1) Sample pretreatment:

Serum (plasma) samples: Use directly (chicken serum (plasma) has high AKP activity and generally needs to be diluted 5 or 10 times with physiological saline before testing; for other species, a preliminary test can be performed before testing) .

Tissue sample: Accurately weigh the tissue to be tested, add physiological saline at a ratio of weight (g):volume (mL) = 1:9, mechanically homogenize under ice-water bath conditions, centrifuge at 2500 rpm for 10 minutes, and take the supernatant for testing (the protein concentration of the supernatant needs to be determined, and protein assay kits are available

from our company).

Cells or culture medium: Refer to Appendix I.

3) Operation Table:

	blank well	standard well	sample well
Double-distilled water (μL)	5		
0.1 mg/mL phenol standard solution (μL)		5	
Sample to be tested (μL)			5
Reagent 1 (μL)	50	50	50
Reagent 2 (μL)	50	50	50
Gently shake the orifice plate to mix thoroughly , and react at 37°C for 15 minutes.			
Reagent 3 (μL)	150	150	150
Gently shake the plate to mix, and measure the absorbance of each well using a microplate reader at a wavelength of 520 nm .			

6. Technical Parameters

Project number	Indicator Name	Indicator Requirements
1	blank well	≤0.150
2	Intra-batch CV	≤ 3%
3	Inter-batch CV	≤ 5%
4	recovery rate	98%
5	Linear range: 0–60 King's units/100 mL	R^2 =0.9999

7. Calculation Formula

1) Liquid sample calculation method: (Applicable to calculations for liquid samples such as culture medium, serum, and plasma)

Definition: One King's unit is defined as the production of 1 mg of phenol from 100 mL of serum or liquid reacting with a matrix at 37°C for 15 minutes.

Calculation formula:

$$\underset{(King'sunit/100mL)}{Liquid\ Sample\ AKP\ Activity} = \frac{A_{sample} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \times 100 \times N$$

$C_{standard}$: Concentration of phenol standard solution, 0.1 mg/mL;

N: Dilution factor of sample before testing.

2) Tissue calculation formula: (Applicable to calculations for cultured cells, tissues, and other related samples)

Definition: One King's unit is defined as the production of 1 mg of phenol per gram of tissue protein when it reacts with the matrix at 37°C for 15 minutes .

Calculation formula:

$$\underset{(King's\ Unit/gprot)}{AKP\ Activity\ of\ Tissue/Cell\ Samples} = \frac{A_{sample} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \div Cpr$$

$C_{standard}$: Concentration of phenol standard solution, 0.1 mg/mL;

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

8. Calculation Examples

Example 1: 5 μL of human serum was taken and AKP was measured according to the operating

table. The absorbance of the blank well was 0.0233, the absorbance of the standard well was 0.2603, and the absorbance of the measurement well was 0.1585. The calculations are as follows:

$$\begin{aligned} AKP\ Activity\ of\ Serum \\ (King's\ Unit/100mL) \end{aligned} = \frac{0.1585 - 0.0233}{0.2603 - 0.0233} \times 0.1 \times 100 \times 1$$
$$= 5.7046\ King's\ Unit/100mL$$

Example 2: Rat serum was diluted 1:1 with physiological saline, and 5 μL was taken for AKP measurement according to the operation table. The absorbance of the blank well was 0.0233, the absorbance of the standard well was 0.2603, and the absorbance of the measurement well was 0.2694. The calculation is as follows:

$$\begin{aligned} AKP\ Activity\ of\ Serum \\ (King's\ Unit/100mL) \end{aligned} = \frac{0.2694 - 0.0233}{0.2603 - 0.0233} \times 0.1 \times 100 \times 2$$
$$= 20.7679\ King's\ Unit/100mL$$

Example 3: 5 μL of 2% rat liver tissue homogenate was used for AKP determination. The absorbance of the blank tube was 0.0233, the standard absorbance was 0.2603 , and the measured absorbance was 0.0629 . Simultaneously, the protein concentration of this 2% rat liver tissue homogenate was determined to be 3.528×10^{-3} gprot/mL. The calculations are as follows:

$$\begin{aligned} AKP\ Activity\ of\ Rat\ Liver\ Tissue \\ (King's\ Unit/gprot) \end{aligned} = \frac{0.0629 - 0.0233}{0.2603 - 0.0233} \times 0.1 \div (3.528 \times 10^{-3})$$
$$= 4.7361\ King's\ Unit/gprot$$

Example 4: 5 μL of 10% tilapia liver tissue homogenate was used for AKP determination. The absorbance of the blank tube was 0.0233, the standard absorbance was 0.2603 , and the measured absorbance was 0.1765 . Simultaneously, the protein concentration of this 10% tilapia liver tissue homogenate was determined to be 4.1386×10^{-3} gprot/mL. The calculations are as follows:

$$\begin{aligned} AKP\ Activity\ of\ Tilapia\ Tissue \\ (King's\ Unit/gprot) \end{aligned} = \frac{0.1765 - 0.0233}{0.2603 - 0.0233} \times 0.1 \div (4.138628 \times 10^{-3})$$
$$= 15.6191\ King's\ Unit/gprot$$

9. Precautions

1) If the microplate reader you are using does not have this wavelength, you can use a similar wavelength of 510nm or 530nm, and the measurement results of 510nm wavelength are better than those of 530nm.
2) Since the sample volume is relatively small, it is recommended to place the pipette tip close to the bottom of the ELISA plate and add the sample slowly, moving the pipette tip up as you add the sample to ensure that the amount of sample remaining on the pipette tip is minimal and to reduce errors in sample addition.
3) The added reagent is close to an aqueous solution, so it has very little adhesion to the pipette tip. However, care should still be taken when adding the reagent, and the speed should not be too fast to avoid splashing it out of the well.
4) If adding samples or reagents near the wall, they should be close to the bottom. The amount of reaction solution in the first part of the treatment is relatively small. If the sample is added close to the top, some of it will stick to the top of the enzyme label well, resulting in incomplete reaction.
5) Since the wells of the enzyme label are relatively small, the mixing force should be moderate.

Too vigorous a mixing motion may cause the liquid to splash out, while too slow a mixing motion will result in insufficient mixing. First, gently shake the liquid on the well wall to let it fall down, and then shake it back and forth and side to side.

6) There may be differences in the initial absorbance of ELISA plates. It is best to measure the initial absorbance at the corresponding wavelength before use and then add the sample for measurement.

7) This kit is for research and laboratory use only.

8) Before the formal experiment, two samples with the largest expected difference need to be taken for preliminary testing. If the enzyme activity is too high, it can be diluted and measured. The blank A value should be controlled at around 0.2 or 0.3. If the enzyme activity is low, it can be measured directly or the sample size can be doubled before measurement.

Appendix I: AKP Measurement in Culture Medium and Cultured Cells

1. Sample Pretreatment:

1) Cell culture medium: Take the culture medium, centrifuge at 1000-1500 rpm for 10 minutes, and take the supernatant for testing.

2) Pretreatment of cultured cells:

① Collection of cultured cells:

Suspension culture of cells : Cell pellet can be collected directly by centrifugation (1000 rpm, centrifuge for 10 minutes, discard the supernatant and keep the cell pellet).

For adherent cells: Aspirate the supernatant, or scrape the cells directly using a cell scraper; alternatively, digest with 0.25% trypsin at room temperature for 2-3 minutes, add culture medium to stop digestion, gently pipette to remove all liquid, transfer to an EP tube, centrifuge at 1000 rpm for 10 minutes, discard the supernatant, and keep the cell pellet . Add 1 mL of PBS, gently pipette, centrifuge again at 1000 rpm for 10 minutes, discard the supernatant, and keep the cell pellet for later use. If not performing this immediately, the cell pellet can be cryopreserved at the lowest possible temperature.

② Disruption of cultured cells:

Grinding and disruption: Add a certain amount of buffer (0.3-0.5 mL for 10^6 cells) to the cell pellet (the buffer can be PBS or physiological saline), grind in an ice-water bath for 3-5 minutes using a manual glass homogenizer, or grind in an ice-water bath for 3 minutes using an electric grinder before testing;

Sonication disruption: Add a certain amount of buffer solution (0.3-0.5 mL for 10^6 cells) to the cell pellet (PBS or physiological saline can be used), ensuring the ultrasound probe is submerged in the liquid. Use 300W power, in an ice-water bath, sonicating once every 3-5 seconds, with a 4-time interval (approximately 30 seconds between each sonication).

Chemical lysis: For adherent cells, the supernatant can be directly aspirated, and a certain amount of lysis buffer (enough to completely cover the cells) can be added directly to the well plate or flask. Lysis is carried out for 30-40 minutes (the cell fragmentation can be observed under a microscope), and then the cells are aspirated for analysis using a micropipette. The cells can be diluted with physiological saline or PBS as needed.

2. Procedure: (First, dilute the **0.1 mg/mL phenol standard solution with distilled water five times (1:4) to obtain a 0.02 mg/mL phenol standard solution, and** then follow the procedure in the table below.)

	blank well	Standard well	Sample well
Double-distilled water (μL)	30		
0.02 mg/mL phenol standard solution (μL)		30	
Sample to be tested (μL)			30
Reagent 1 (μL)	50	50	50
Reagent 2 (μL)	50	50	50
Mix thoroughly and bathe in a 37°C water bath for 15 minutes.			
Reagent 3 (μL)	150	150	150
Gently shake the plate to mix, and measure the absorbance of each well using a microplate reader at a wavelength of 520 nm.			

3. Calculation formulas and examples:

1) Example of calculating culture medium:

Example: 30 μL of cell culture medium was taken and AKP was measured according to the operating table. The absorbance of the blank well was 0.0263, the absorbance of the standard well was 0.3172, and the absorbance of the measurement well was 0.2698. The calculation is as follows:

$$AKP\ Activity\ in\ Culture\ Medium\ (King's\ Unit/100mL) = \frac{0.2698 - 0.0263}{0.3172 - 0.0263} \times 0.02 \times 100$$
$$= 1.6741\ King's\ Unit/100mL$$

2) Example of calculations for cultured cells:

Example: Osteoblasts cultured in 12-well plates were subjected to lysis buffer (1% Triton X-100) after the culture medium was aspirated. The cells were then covered with 0.2 mL of lysis buffer. After lysis, 30 μL of the lysate was taken and AKP was measured according to the procedure. The absorbance of the blank well was 0.0263, the absorbance of the standard well was 0.3172, and the absorbance of the measurement well was 0.1465. Simultaneously, the protein concentration was determined using the BCA method to be 0.8546 × 10^{-3} gprot /mL. The calculations are as follows:

$$AKP\ Activity\ of\ Cultured\ Cells\ (King's\ Unit/gprot) = \frac{0.1465 - 0.0263}{0.3172 - 0.0263} \times 0.02 \div (0.8546 \times 10^{-3})$$
$$= 9.67\ King's\ Unit/gprot$$

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Appendix II: AKP Standard Curve Construction

1. Sample Pretreatment:

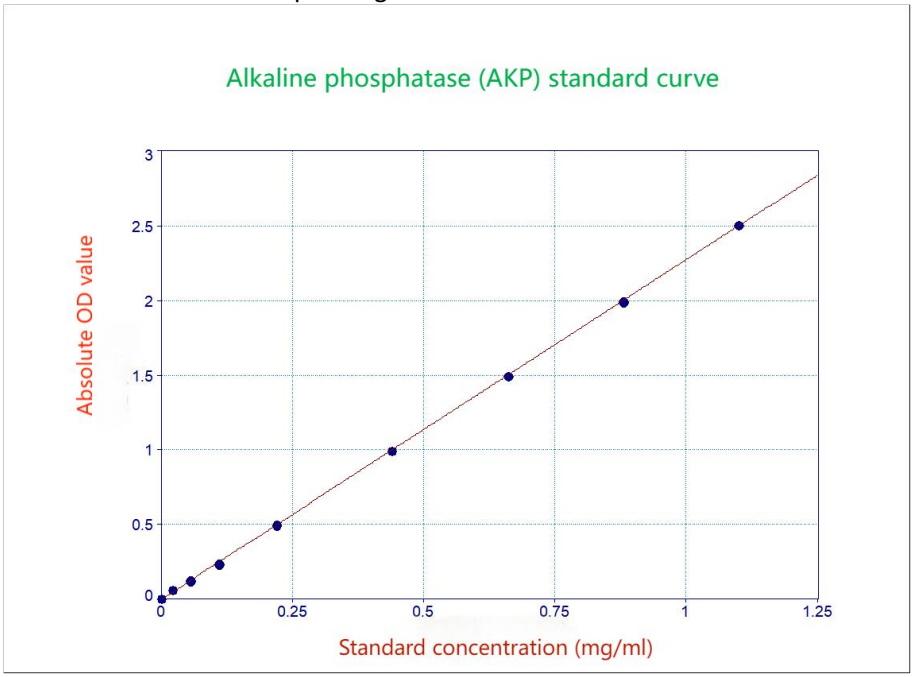
The standard stock solution was diluted with distilled water at concentrations of 50 , 20 , 10 , 5 , 2.5 , and 1.25 times (concentrations of 0.022 mg/mL, 0.055 mg/mL, 0.11 mg/mL, 0.22 mg/mL, 0.44 mg/mL, 0.88 mg/mL, and 1.1 mg/mL).

2. Operation Table:

	blank well	Standard well
Double-distilled water (μL)	5	
Phenol standard solutions of different concentrations (μL)		5
Reagent 1 (μL)	50	50
Reagent 2 (μL)	50	50
Gently shake the orifice plate to mix thoroughly , and react at 37°C for 15 minutes.		
Reagent 3 (μL)	150	150
Gently shake the plate to mix , and measure the absorbance value A at a wavelength of 520 nm using an ELISA reader.		

3. Measurement Results:

A standard curve is plotted with the measured absolute absorbance (absolute OD value) as the ordinate and the corresponding standard concentration unit as the abscissa.



Users can skip the standard curve step and simply follow the instructions in the operation table to input the calculation formula; this will not affect the results.