



## **PRODUCT INFORMATION & MANUAL**

### **Alkaline Phosphatase Activity Assay Kit (Colorimetric) *NBP3-24465***

For research use only.  
Not for diagnostic or therapeutic  
procedures.

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# Alkaline Phosphatase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24465

Method: Colorimetric method

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

Instrument: Spectrophotometer

Sensitivity: 0.2 King unit/100 mL

Detection range: 0.2-55.6 King unit/100 mL

Average intra-assay CV (%): 2.1

Average inter-assay CV (%): 5.6

Average recovery rate (%): 99

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

## General information

### ▲ Intended use

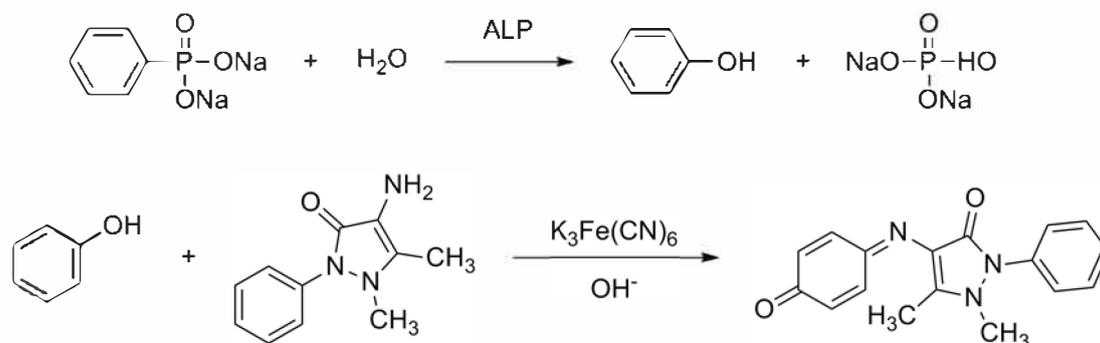
This kit can be used for detection of ALP activity in serum, plasma, urine, tissue and cells sample.

### ▲ Background

Alkaline phosphatase (ALP) is a group of cytomembrane-related enzymes with hydrolysis and transfer activity, acting on a variety of phosphate substrates. ALP is a homologous dimerase and each catalytic site contains three metal ions. There are four isozymes in humans: tissue nonspecific ALP, intestinal ALP, placental ALP and genital cell ALP.

### ▲ Detection principle

Alkaline phosphatase decompose benzene disodium phosphate to produce free phenol and phosphoric acid. Phenol react with 4-aminopyrline in alkaline solution and oxidizes with potassium ferricyanide to form red quinone derivative. The enzyme activity can be calculated indirectly by measuring the OD value.



## ▲ Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	60 mL × 1 vial	2-8°C , 12 months, shading light
Reagent 2	Substrate Solution	60 mL × 1 vial	2-8°C , 12 months, shading light
Reagent 3	Chromogenic Agent	60 mL × 3 vials	2-8°C , 12 months, shading light
Reagent 4	0.5 mg/mL Phenol Standard	1.5 mL × 1 vial	2-8°C , 12 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

### 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 point of the assay

Add reagent 3 immediately after incubating at 37 °C for 15 min.

## Pre-assay preparation

### ▲ Reagent preparation

Preparation of 0.1 mg/mL phenol standard application solution

Dilute reagent 4 with double distilled water at a ratio of 1:4. Prepared the fresh solution before use.

### ▲ Sample preparation

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

### Sample requirements

1. The samples should not contain EDTA, citrate, oxalate, or high concentrations of inorganic phosphorus.
2. Glucose, aminosugar and benzidine will inhibit the activity of ALP.

### ▲ 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 (0.2-55.6 King unit/100 mL).

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

Sample type	Dilution factor
Human serum	1
Human urine	1
Rat serum	1
Cellular supernatant	1
10% Mouse kidney tissue homogenization	30-50
10% Mouse liver tissue homogenization	1
HePG2 cell	1
10% Mouse brain tissue homogenization	1

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

# Assay protocol

## ▲ Detailed operation steps

1. **Blank tube:** add 50  $\mu\text{L}$  of double distilled water into a 5 mL EP tube.  
**Standard tube:** add 50  $\mu\text{L}$  of 0.1 mg/mL phenol standard application solution into a 5 mL EP tube.  
**Sample tube:** add 50  $\mu\text{L}$  of sample into a 5 mL EP tube.
2. Successively add 500  $\mu\text{L}$  of reagent 1 and 500  $\mu\text{L}$  of reagent 2 respectively and mix fully with a vortex mixer.
3. Incubate at 37°C for 15 min, then add 1500  $\mu\text{L}$  of reagent 3 immediately, mix fully.
4. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 520 nm with 0.5 cm optical path quartz cuvette.

## ▲ Summary 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°C for 15 min.			
Reagent 3 (μL)	1500	1500	1500
Mix fully, then set the spectrophotometer to zero and measure the OD values of each tube.			

## ▲ Calculation

### 1. For serum (plasma) and other liquid samples

#### (1) Definition

100 mL of sample reacts with the substrate at 37°C for 15 min to produce 1 mg of phenol that is defined as 1 King unit.

#### (2) Calculation formula

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

## 2. For tissue samples

### (1) Definition

1 g of tissue protein reacts with the substrate at 37°C for 15 min to produce 1 mg of phenol that is defined as 1 King unit.

### (2) Calculation formula

$$\text{ALP activity (King unit/gprot)} = \frac{\Delta A_1}{\Delta A_2} \times m \div (\text{Cpr} \times V) \times f$$

#### **Note:**

$\Delta A_1$ :  $OD_{\text{Sample}} - OD_{\text{Blank}}$

$\Delta A_2$ :  $OD_{\text{Standard}} - OD_{\text{Blank}}$

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

$V_1$ : The volume of sample in definition, 100 mL

f: Dilution factor of sample before test.

## Appendix I Data

### ▲ Example analysis

Take 50  $\mu$ L of rat serum, carry the assay according to the operation steps. The results are as follows:

The average OD value of the sample is 0.345, the average OD value of the blank is 0.020, the average OD value of the standard is 0.270, and the calculation result is:

$$\begin{aligned} \text{ALP activity(King unit/100 mL)} &= (0.345 - 0.020) \div (0.270 - 0.020) \times 0.005 \times (100 \div 0.05) \\ &= 13 \text{ (King unit/100 mL)} \end{aligned}$$

## 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.

### ▲ Tissue

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C 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 ( $\mu\text{L}$ ) = 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. If not detected on the same day, the cells sample (without homogenization) can be stored at  $-80^\circ\text{C}$  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 ( $1\text{ mm}^3$ ), 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).

## Appendix III References

1. Millán J L. Alkaline Phosphatases. *Biochemical Society Transactions*, 1990, 18(2):178-180.
2. Millán J L. Alkaline Phosphatases: Structure, substrate specificity and functional relatedness to other members of a large superfamily of enzymes. *Purinergic Signalling*, 2009, 2(2): 335-341.
3. Fernandez N J, Kidney B A. Alkaline phosphatase: beyond the liver. *Veterinary Clinical Pathology*, 2010, 36(3): 223-233.