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## Technical Manual

### Alkaline Phosphatase (ALP) Activity Assay Kit

- Catalogue Code: MAES0081
- Size: 100 Assays
- Research Use Only

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## 1. Key features and Sample Types

### Detection method:

Colorimetric method

### Specification:

100 Assays

### Range:

0.2-55.6 King unit/100 mL

### Sensitivity:

0.2 King unit/100 mL

### Storage:

2-8°C for 6 months

### Expiry:

See Kit Label

### Experiment Notes:

This kit is for **research use only**.

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

## 2. 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

## 3. Intended Use

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

## 4. 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.

## 5. Kit components & storage

Item	Specification	Storage
<b>Buffer Solution</b>	60 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
<b>Substrate Solution</b>	60 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
<b>Chromogenic Agent</b>	60 mL × 3 vials	2-8°C, 6 months, avoid direct sunlight
<b>Phenol Standard (0.5 mg/mL)</b>	1.5 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight

### Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Spectrophotometer (520 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

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## 6. Assay Notes:

After incubating at 37°C for 15 min, add chromogenic agent quickly.

## 7. Reagent preparation:

1. Bring all reagents to room temperature before use.
2. Preparation of phenol standard application solution (0.1 mg/mL): Dilute phenol standard (0.5 mg/mL) with double distilled water at a ratio of 1:4. Prepared the fresh solution before use.

## 8. Sample Preparation

### Sample requirements:

Samples should not contain EDTA, citrate, oxalate, high concentration of inorganic phosphorus.

Glucose, amino sugar, benzidine in the sample will inhibit the activity of ALP.

### 1. Serum sample:

Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample 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.

### 2. Plasma sample:

Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g 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.

### 3. 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.

### 4. Cell sample:

Collect the cells 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 ( $2 \times 10^6$ ): PBS (0.01 M, pH 7.4) or 0.9% NaCl ( $\mu\text{L}$ ) = 1: 200. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

## 5. Tissue sample:

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 PBS (0.01 M, pH 7.4) or 0.9% NaCl (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. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

## Sample Notes:

The concentration should be determined before performing the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

## Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. 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 plasma	1
Human urine	1
Rat serum	1
Cells culture supernatant	1
10% Mouse kidney tissue homogenate	30-50
0% Mouse liver tissue homogenate	1
10% Mouse brain tissue homogenate	1
HepG2 cells	1

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

## 9. Assay Protocol

**Ambient Temperature:** 25-30°C

**Optimum detection wavelength:** 520 nm

## 10. 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 buffer solution and 500  $\mu\text{L}$  of substrate solution respectively and mix fully with a vortex mixer.
3. Incubate at 37°C for 15 min, then add 1500  $\mu\text{L}$  of chromogenic agent 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.

### Operation Table

	Blank tube	Standard tube	Sample tube
Double distilled water ( $\mu\text{L}$ )	50		
0.1 mg/mL Phenol standard application solution ( $\mu\text{L}$ )		50	
Sample ( $\mu\text{L}$ )			50
Buffer solution ( $\mu\text{L}$ )	500	500	500
Substrate solution ( $\mu\text{L}$ )	500	500	500
Mix fully and incubate at 37°C for 15 min.			
Chromogenic agent ( $\mu\text{L}$ )	1500	1500	1500
Mix fully immediately, then 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.			

## 11. Calculations

### 1. Serum (plasma) and other liquid sample:

**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.

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

### 2. Tissue and cell sample:

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

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

$\Delta A_1$ :  $OD_{\text{sample}} - OD_{\text{blank}}$

$\Delta A_2$ :  $OD_{\text{standard}} - OD_{\text{blank}}$

**m**: Phenol content of standard tube, 0.005 mg

**C<sub>pr</sub>**: Protein concentration of tested sample, gprot/mL

**V**: The volume of sample, 0.05 mL

**V<sub>1</sub>**: The volume of sample in definition, 100 mL

**f**: Dilution factor of sample before test.

## 12. Performance Characteristics

Detection Range	0.2-55.6 King unit/100 mL
Sensitivity	0.2 King unit/100 mL
Average recovery rate (%)	99
Average inter-assay CV (%)	5.6
Average intra-assay CV (%)	2.1

### Analysis

Take 50 µL of rat serum, carry the assay according to the operation table.

#### 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:

$$\text{ALP activity (King unit/100 mL)} = \frac{0.345-0.020}{0.270-0.020} \times 0.005 \times \frac{100}{0.05} = 13.01 \text{ (King unit/100 mL)}$$

## Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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