# Technical Manual

# Alkaline Phosphatase (ALP) Colorimetric Assay Kit (PNPP method)

- Catalogue Code: MAES0023
- Size: 96T
- Research Use Only

# 1. Key features and Sample Types

### Detection method:

Colorimetric method

### Specification:

96T

### Range:

0.27-50.8 U/L

### Sensitivity:

0.27 U/L

### Storage:

-20°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 to measure alkaline phosphatase (ALP) activity in animal tissue, serum (plasma) and cell samples.

## 4. Detection Principle

Under alkaline conditions, alkaline phosphatase catalyzes the hydrolysis of p-nitrobenzene phosphate disodium to produce p-nitrophenol and phosphoric acid. Under strong alkaline conditions, p-nitrophenol is bright yellow and has a maximum absorption peak at 405 nm. Therefore, the activity of ALP can be calculated by measuring the OD value at 405 nm.

## 5. Kit components & storage

| Item | Specification | Storage |
|---|---|---|
| **Buffer Solution** | 60 mL×1 vial | -20°C, 6 months |
| **Substrate** | 2 vials Lyophilized | -20°C, 6 months, away from direct sunlight |
| **p-Nitrophenol Standard Solution (10 mmol/L)** | 0.4 mL×1 vial | -20°C, 6 months, away from direct sunlight |
| **Stop Solution** | 12 mL × 1 vial | -20°C, 6 months |
| **Microplate** | 96 wells | No requirement |
| **Plate Sealer** | 2 pieces | |

### Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (400-415 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Normal Saline (0.9% NaCl)

## 6. Assay Notes:

Substrate working solution and standard should be stored away from direct sunlight.

## 7. Reagent preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **substrate working solution:** Dissolve substrate with 3 mL of buffer solution. The prepared solution can be stored at -20°C with shading light for 24 hours.

3. Preparation of **500 μmol/L standard:** Mix the 10 mmol/L standard and buffer solution at the ratio of 1:19. Prepare the fresh needed amount before use and store away from direct sunlight for detection.

## 8. Sample Preparation

1. **Serum (Plasma):** Detect the sample directly.

2. **Tissue Sample:** Weigh the tissue. Add normal saline (0.9% NaCl) in a weight (g): volume (mL) ratio of 1:9. Homogenize mechanically in an ice bath to fully break the cells. Centrifuge at 10000 g for 10 min at 4˚C and collect the supernatant for measurement. Meanwhile, determine the protein concentration of the supernatant.

3. **Cell Sample:** Add normal saline (0.9% NaCl) at a ratio of cell number ($3*10^8$): 0.9% NaCl (μL) = 1:200-400.

4. **Sample Requirements:** The Sample Solution should not include inhibitors of alkaline phosphatase, such as EDTA, fluoride ion and citrate.

### Sample Notes:

The concentration should be determined before preforming 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 (0.27-50.8 U/L).

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

| Sample Type: | Dilution Factor |
|---|---|
| Human serum | 10-20 |
| Mouse serum | 20-30 |
| Rat serum | 10-20 |
| Mouse plasma | 10-20 |
| HepG2 supernatant | 8-12 |
| 10% Rat kidney tissue homogenate | 400-1200 |
| 10% Mouse liver tissue homogenate | 10-15 |
| 10% Rat lung tissue homogenate | 50-100 |
| 10% Mouse brain tissue homogenate | 30-50 |
| Human urine | 1 |

**Note:** The diluent is buffer solution

## 9. Assay Protocol

**Ambient Temperature:** 25-30°C

**Optimum detection wavelength:** 405 nm

### Plate Set Up:

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | A | A | S0 | S8 | S16 | S24 | S32 | S40 | S48 | S56 | S64 | S72 |
| **B** | B | B | S1 | S9 | S17 | S25 | S33 | S41 | S49 | S57 | S65 | S73 |
| **C** | C | C | S2 | S10 | S18 | S26 | S34 | S42 | S50 | S58 | S66 | S74 |
| **D** | D | D | S3 | S11 | S19 | S27 | S35 | S43 | S51 | S59 | S67 | S75 |
| **E** | E | E | S4 | S12 | S20 | S28 | S36 | S44 | S52 | S60 | S68 | S76 |
| **F** | F | F | S5 | S13 | S21 | S29 | S37 | S45 | S53 | S61 | S69 | S77 |
| **G** | G | G | S6 | S14 | S22 | S30 | S38 | S46 | S54 | S62 | S70 | S78 |
| **H** | H | H | S7 | S15 | S23 | S31 | S39 | S47 | S55 | S63 | S71 | S79 |

**Note:** A-H, standard wells; S0, control wells; S1-S79, sample wells.



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# 10. Operation Steps

## The preparation of standard curve

Dilute 500 μmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 500, 400, 320, 240, 160, 80, 40, 0 μmol/L.

## The measurement of samples

1. **Standard well:** Add 50 μL of standard solution with different concentrations to the wells.
   **Sample well:** Add 50 μL of sample to the wells.
   **Control well:** Add 50 μL of buffer solution into the control wells.

2. Add 50 μL of substrate working solution into the control wells and sample wells.
   Add 50 μL of buffer solution into the standard wells.

3. Incubate at 37˚C for 10 min.

4. Add 100 μL of stop solution into each well.

5. Mix fully with microplate reader for 5 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)** | 50 | | |
| **Sample (μL)** | | 50 | |
| **Buffer solution (μL)** | 50 | | 50 |
| **Substrate working solution (μL)** | | 50 | 50 |
| Incubate at 37℃ for 10 min. | | | |
| **Stop solution (μL)** | 100 | 100 | 100 |
| Mix fully with microplate reader for 5 s. Measure the OD values of each well at 405 nm with microplate reader. | | | |

## 11. Calculations

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 catalyze the substrate at 37°C is defined as 1 activity unit.

$$\text{ALP activity (U/L)} = (\Delta A\text{-}b) \div a \div T \times f$$

**2**. **Tissue and cells sample:**

**Definition:** The amount of 1 μmol p-nitrophenol produced by 1 g sample protein per minute catalyze the substrate at 37°C is defined as 1 activity unit.

$$\text{ALP activity (U/gprot)} = (\Delta A - b) \div a \div T \times f \div C_{pr}$$

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 sample, gprot/L.

## 12. Performance Characteristics

| | |
|---|---|
| **Detection Range** | 0.27-50.8 U/L |
| **Sensitivity** | 0.27 U/L |
| **Average inter-assay CV (%)** | 4.6 |
| **Average intra-assay CV (%)** | 1.2 |

### Analysis

For human serum, dilute human serum with buffer solution for 10 times, take 50 μL of diluted samples, carry the assay according to the operation table. The results are as follow.

**The results are as follows:**

standard curve: y = 0.0027 x – 0.0051, the OD value of the control is 0.099, the OD value of the sample is 0.544, and the calculation result is:

$$\boldsymbol{ALP\ activity}\left(\frac{\boldsymbol{U}}{\boldsymbol{L}}\right) = (\mathbf{0.544} - \mathbf{0.099} + \mathbf{0.0051}) \div \mathbf{0.0027} \div \mathbf{10} \times \mathbf{10}$$

$$= \mathbf{166.70}\ \boldsymbol{U/L}$$



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## Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid 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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## Notes:



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