# Acid Phosphatase Assay Kit

Acid Phosphatase Assay Kit is a detection kit for the quantification of Acid Phosphatase Activity in urine, serum, plasma, tissue extracts, cell lysate and cell culture supernatants.

Catalog number: ARG82014

Package: 96 wells

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## INTRODUCTION

Acid phosphatase (EC 3.1.3.2) is a phosphatase, a type of enzyme, used to free attached phosphoryl groups from other molecules during digestion. It can be further classified as a phosphomonoesterase. Acid phosphatase is stored in lysosomes and functions when these fuse with endosomes, which are acidified while they function; therefore, it has an acid pH optimum. This enzyme is present in many animal and plant species.

Different forms of acid phosphatase are found in different organs, and their serum levels are used to evaluate the success of the surgical treatment of prostate cancer. In the past, they were also used to diagnose this type of cancer.

It's also used as a cytogenetic marker to distinguish the two different lineages of Acute Lymphoblastic Leukemia (ALL): B-ALL (a leukemia of B Lymphocytes) is Acid-Phosphatase negative, T-ALL (originating instead from T Lymphocytes) is acid-phosphatase positive. [Provide by Wikipedia: Acid phosphatase]

## PRINCIPLE OF THE ASSAY

This Acid Phosphatase Assay Kit is a simple colorimetric assay that measures the amount of Acid Phosphatase (ACP) present in urine, serum, plasma, tissue extracts, cell lysate and cell culture supernatants. The assay is based on the enzyme driven reaction. The assay is initiated with the enzymatic hydrolysis of the disodium phenyl phosphate by acid phosphatase. The enzyme catalysed reaction products can be measured at a colorimetric readout at 510 nm. Samples and standards are read with a plate reader. The concentration of ACP in the samples is then determined by comparing the O.D. 510 nm absorbance of samples to the standard curve.

## MATERIALS PROVIDED & STORAGE INFORMATION

| Component | Quantity | Storage information |
|---|---|---|
| 96 Well microplate | 1 plate | RT |
| Assay Buffer | 4 x 30 mL (ready to use) | 4°C |
| Reaction Buffer | 4 mL (ready to use) | 4°C (protect from light) |
| Substrate (lyophilized) | 1 vial | 4°C (protect from light) |
| Dye Reagent I (lyophilized) | 1 vial | 4°C (protect from light) |
| Dye Reagent II (lyophilized) | 1 vial | 4°C (protect from light) |
| Standards (lyophilized) | 1 vial | 4°C |
| Positive Control (lyophilized) | 1 vial | -20°C |
| Plate sealer | 3 ea | RT |
| Technical Manual | 1 ea | RT |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 510 nm
- Centrifuge
- Mortar
- Deionized or Distilled water
- Ice

- Pipettes and pipette tips
- Multichannel micropipette reservoir

## TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

## SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

**Cell and bacteria samples:** Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mL of Assay Buffer for $5\times10^6$ cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 8,000 x g for 10 minutes at 4°C, take the supernatant into a new centrifuge tube and keep it on ice for detection.

**Tissue samples:** Weigh out 0.1 g tissue, homogenize with 1 mL Assay Buffer on ice, centrifuged at 8,000 x g for 10 minutes at 4°C, take the supernatant into a new centrifuge tube and keep it on ice for detection.

**Plasma:** Collect blood with EDTA, heparin or citrate and centrifuge at 2,000 x g and 4°C for 10 minutes. The supernatant should be assayed directly or diluted as necessary in 1X Assay Buffer.

**Serum:** Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2,500 x g for 20 minutes. The supernatant should be assayed directly or diluted as necessary in Assay Buffer.

**Urine and Saliva:** To remove insoluble particles, centrifuge at 10,000 x g for 10 minutes at 4°C. The supernatant should be assayed directly or diluted as necessary in Assay Buffer.

**Note:**

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum / plasma sample.

## REAGENT PREPARATION

- **Substrate:** Add 4 mL of distilled water to dissolve before use.
- **Dye Reagent I:** Add 9 mL of distilled water to dissolve before use.
- **Dye Reagent II:** Add 2 mL of distilled water to dissolve before use.
- **Positive Control:** Add 1 mL of Assay Buffer to dissolve before use and mix well.
- **Standards:** add 1 ml distilled water to dissolve, then add 40 µL of standard into 960 µL of distilled water, the concentration will be 4 mmol/L. Use the 4 mmol/L Standards to prepare a series of standards according to the Table below.

| Standard tube | Final Lysine conc. (mmol/L) | Volume of distilled water (µL) | Volume of 4 mmol/L Standards (µL) |
|---|---|---|---|
| S1 | 4 | 0 | 500 |
| S2 | 2 | 250 | 250 of S1 |
| S3 | 1 | 250 | 250 of S2 |
| S4 | 0.5 | 250 | 250 of S3 |
| S5 | 0.25 | 250 | 250 of S4 |
| S6 | 0.125 | 250 | 250 of S5 |
| S7 | 0.0625 | 250 | 250 of S6 |
| S0 | 0 | 250 | 0 |

## ASSAY PROCEDURE

Each Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add **10 µL** of **samples, Blank, Positive Control** or serial **diluted Standards** into 96-well microplate.
2. Add **40 µL** of **Reaction Buffer** into each well.
3. Add **40 µL** of **Substrate** into each well.
4. Incubate for **15 minutes** at **37°C** in the oven.
5. Add **90 µL** of **Dye Reagent I** into each well.
6. Add **20 µL** of **Dye Reagent II** into each well.
7. Mix well, and incubate for **10 minutes** at **room temperature.**
8. Read the plate with a microplate reader at **510 nm**.

## CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of Standards, Control, Blank and samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Unit Definition: One unit of Acid Phosphatase activity is defined as the enzyme generates 1 nmol phenol per minute.
5. According to the protein concentration of sample:

ACP (U/mg)

$= \{[(C_{Standaard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / (V_{Sample} / C_{Protein})\} / T$

$= [266.67 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / C_{Protein}$

6. According to the weight of sample:

ACP (U/g)

$= \{[(C_{Standaard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / (V_{Sample} \times W / V_{Assay})\} / T$

$= [266.67 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / W$

7. According to the volume of serum or plasma:

ACP (U/mL)

$= \{[(C_{Standaard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / V_{Sample}\} / T$

$= 266.67 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$

Note:

$C_{Protein}$: the protein concentration of sample, mg/mL;

W: the weight of sample, g;

$C_{Standard}$: the concentration of standard, 4 mmol/L = 4000 nmol/mL;

$V_{Standard}$: the volume of standard, 0.01 mL;

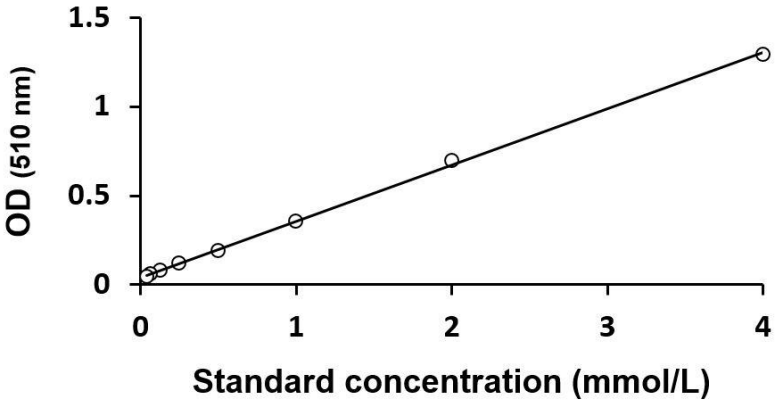
$V_{Sample}$: the volume of sample, 0.01 mL;

$V_{Assay}$: the volume of Assay buffer, 1 mL;

T: the reaction time, 15 minutes.

## EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Acid Phosphatase Kit. One should use the data below for reference only. This data should not be used to interpret actual results.




## QUALITY ASSURANCE

### Sensitivity

0.04 mmol/L