



# Acid Phosphatase Microplate Assay Kit User Manual

**Catalog # ASK1001**

Detection and Quantification of Acid Phosphatase (ACP) Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

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## **I. INTRODUCTION**

Acid phosphatases (ACP) dephosphorylate phosphate groups from phosphate esters under acid conditions. Different acid phosphatase isozymes are found in different organs, and their serum levels are used as a diagnostic for disease in the corresponding organs. Elevated prostatic acid phosphatase levels may indicate the presence of prostate cancer and elevated tartrate-resistant acid phosphatase levels may indicate bone disease.

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.



II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	4 ml x 1	4 °C, keep in dark
Substrate	Powder x 1	4 °C, keep in dark
Dye Reagent I	Powder x 1	4 °C, keep in dark
Dye Reagent II	Powder x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

**Note:**

**Substrate:** add 4 ml distilled water to dissolve before use.

**Dye Reagent I:** add 10 ml distilled water to dissolve before use.

**Dye Reagent II:** add 2 ml distilled water to dissolve before use.

**Standard:** add 1 ml distilled water to dissolve, then add 40 µl standard into 960 µl distilled water, the concentration will be 4 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 510 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer



**IV. SAMPLE PREPARATION**

**1. For cell and bacteria samples**

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay Buffer for  $5 \times 10^6$  cell or bacteria, sonicate (with power 20%, sonication 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

**2. For tissue samples**

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

**3. For serum or plasma samples**

Detect directly, or dilute with Assay Buffer.

**V. ASSAY PROCEDURE**

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	10 $\mu$ l	--	--
Standard	--	10 $\mu$ l	--
Distilled water	--	--	10 $\mu$ l
Reaction Buffer	40 $\mu$ l	40 $\mu$ l	40 $\mu$ l
Substrate	40 $\mu$ l	40 $\mu$ l	40 $\mu$ l
Mix, put it in the oven, 37 °C for 15 minutes.			
Dye Reagent I	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l
Dye Reagent II	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l
Mix, wait for 10 minutes, record absorbance measured at 510 nm.			

**VI. CALCULATION**

**Unit Definition:** One unit of Acid Phosphatase activity is defined as the enzyme generates 1 nmol phenol per minute.

1. According to the protein concentration of sample

$$\begin{aligned} \text{ACP (U/mg)} &= C_{\text{Standard}} \times V_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V_{\text{Sample}} / \\ & C_{\text{Protein}} / T \\ &= 266.67 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{ACP (U/g)} &= C_{\text{Standard}} \times V_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times \\ & W / V_{\text{Assay}}) / T \\ &= 266.67 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

3. According to the volume of serum or plasma

$$\begin{aligned} \text{ACP (U/ml)} &= C_{\text{Standard}} \times V_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V_{\text{Sample}} / T \\ &= 266.67 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

$C_{\text{Protein}}$ : the protein concentration, mg/ml;

$W$ : the weight of sample, g;

$C_{\text{Standard}}$ : the concentration of Standard, 4 mmol/L = 4000 nmol/ml;

$V_{\text{Standard}}$ : the total volume of standard, 0.01 ml;

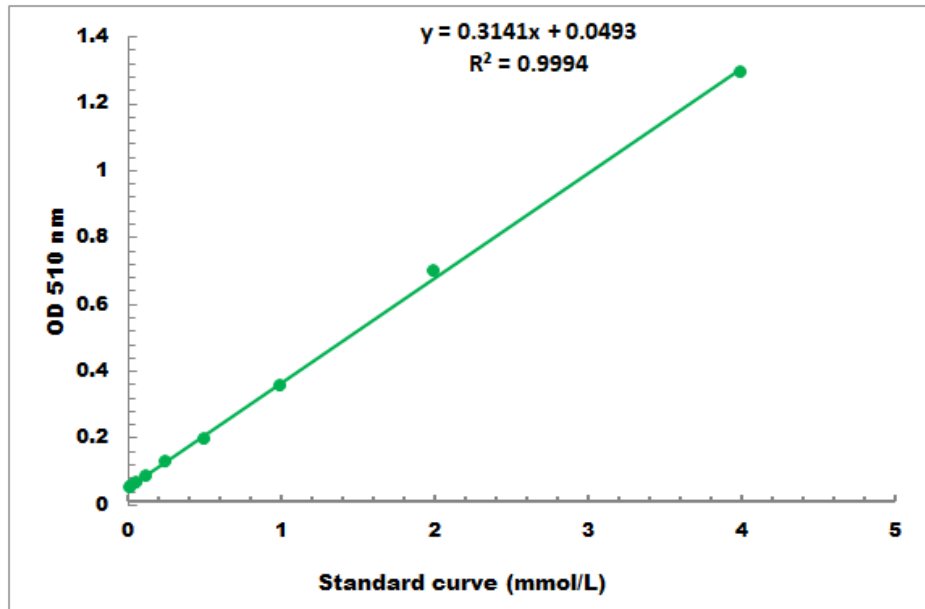
$V_{\text{Sample}}$ : the volume of sample, 0.01 ml;

$V_{\text{Assay}}$ : the volume of Assay buffer, 1 ml;

$T$ : the reaction time, 15 minutes.

**VII. TYPICAL DATA**

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.04 mmol/L - 4 mmol/L