Acid Phosphatase

Microplate Assay Kit

User Manual

Catalog # CAK1001

(Version 1.3E)

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.

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
Positive Control	Powder x 1	-20 °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 9 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 50 µl standard into 950 µl distilled water, the concentration will be 5 mmol/L.

Positive Control: add 1 ml Assay Buffer to dissolve before use, mix.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 510 nm
2. Distilled water
3. Pipettor, multi-channel 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 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 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	Positive Control
Sample	10 µl	--	--	--
Standard	--	10 µl	--	--
Distilled water	--	--	10 µl	--
Positive Control	--	--	--	10 µl
Reaction Buffer	40 µl	40 µl	40 µl	40 µl
Substrate	40 µl	40 µl	40 µl	40 µl
Mix, put it in the oven, 37 °C for 15 minutes.				
Dye Reagent I	90 µl	90 µl	90 µl	90 µl
Dye Reagent II	20 µl	20 µl	20 µl	20 µl
Mix, wait for 10 minutes, record absorbance measured at 510 nm.				

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

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

1. According to the protein concentration of sample

$$ACP\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / C_{Protein} / T$$

$$= 0.333 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$ACP\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 0.333 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the volume of serum or plasma

$$ACP\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.333 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

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

W: the weight of sample, g;

$C_{Standard}$: the concentration of standard, 5 mmol/L = 5 μmol/ml;

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

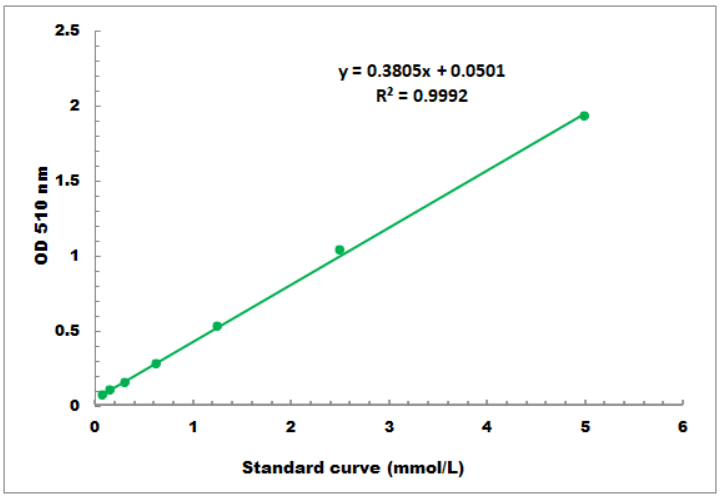
V_{Sample}: the volume of sample, 0.01 ml;

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

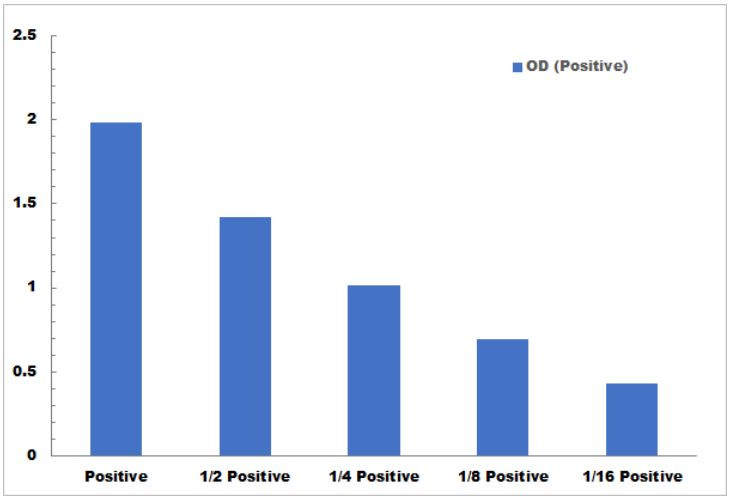
T: the reaction time, 15 minutes.

VII. TYPICAL DATA

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



Detection Range: 0.05 mmol/L - 5 mmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES