Acid Phosphatase Microplate Assay Kit

Catalog # AS0001

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

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic 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 mlx 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 40µlstandard into 960µldistilled water, the concentration will be 4mmol/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 mlAssay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s,repeat 30 times); centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay Buffer on ice, centrifuged at 8000g 4°C for 10minutes, 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 intothe microplate:

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

VI. CALCULATION

Unit Definition: One unit of Acid Phosphatase activity is defined asthe enzyme generates 1 nmolphenol per minute.

1. According to the protein concentration of sample

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

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

2. According to the weight of sample

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

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

3. According to the volume of serum or plasma

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

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

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

W: theweight of sample, g;

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

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

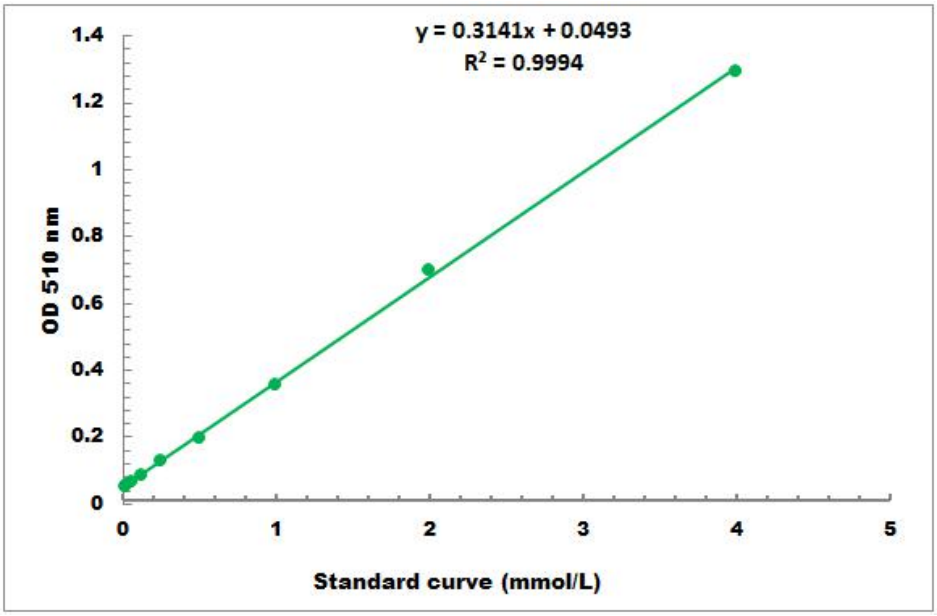
V_{Sample}: the volume of sample, 0.01 ml;

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: 0.04mmol/L -4mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

IX. NOTES