Acid Phosphatase Assay Kit

User Manual

Catalog # MBS822357

Detection and Quantification of Acid Phosphatase Concentrations in Urine, Serum, Plasma, Other biological fluids, Tissue Extracts, Cell Lysate, Cell culture media Samples.

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

I. INTRODUCTION.....2
II. KIT COMPONENTS.....3
III. MATERIALS REQUIRED BUT NOT PROVIDED.....3
VI. SAMPLE PREPARATION.....4
V. ASSAY PROCEDURE.....5
VI. CALCULATION.....6
VII. TECHNICAL SUPPORT.....7
VIII. NOTES.....7

I. INTRODUCTION

Acid phosphatases (AP) 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.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate		
Reagent I	30 ml x 2	4 °C
Reagent II	5 ml x 1	4 °C, keep in dark
Reagent III	10 ml x 1	4 °C, keep in dark
Reagent IV	15 ml x 1	4 °C, keep in dark
Standard	1 ml x 1 (1 mg/ml)	4 °C
Technical Manual	1 Manual	

Note:

Standard: add 9 ml distilled water to dilute as the Standard Solution before use, the concentration is 0.1 mg/ml.

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 (for cell lysis)
8. Timer

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.5ml Reagent I for 2×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10min, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weight ~0.05g tissue, homogenize with 0.5ml Reagent I on ice, centrifuged at 8000g 4 °C for 10min, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent	Experiment	Standard	Blank	Control
Sample	10 ul	--	--	--
Standard Solution	--	10 ul	--	--
Distilled water	--	--	10 ul	--
Reagent I	40 ul	40 ul	40 ul	40 ul
Mix, put it in water bath of 37 °C for 5 minutes.				
Reagent II	40 ul	40 ul	40 ul	40 ul
Mix, put it in water bath of 37 °C for 60 minutes.				
Reagent III	80 ul	80 ul	80 ul	80 ul
Reagent IV	120 ul	120 ul	120 ul	120 ul
Sample	--	--	--	10 ul
Mix, record absorbance measured at 510 nm.				

Notice:

1. Keep Reagent II, Reagent III, Reagent IV in dark.

2. When Reagent IV turn to blue, it is can not use.

3. After add Reagent IV, mix immediately.

4. The sample should be prepared freshly. For serum sample, add 10 mg Disodium Citrate or 5 mg Sodium Acid Sulfate in 1 ml serum, store at 4 °C.

VI. CALCULATION

1. Calculation of AP in cell, tissue sample

AP (U/mg prot) = 0.1 × (OD_{Sample} - $OD_{Control}$) / ($OD_{Standard}$ - OD_{Blank}) / Cpr

Cpr: protein concentration, mg/mL. The protein concentration should be detected separately, you can buy BCA protein concentration assay kit.

2. Calculation of AP in serum sample

AP (U/100ml) = 0.1 × (OD_{Sample} - $OD_{Control}$) / ($OD_{Standard}$ - OD_{Blank}) × 100

VII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online

VIII. NOTES