Acid Phosphatase Assay Kit

Catalog No.: abx096000

Size: 96 tests

Detection Range: 0.2 U/L – 50 U/L

Sensitivity: 0.2 U/L

Storage: Store all components at -20°C for up to 12 months. Store the Substrate and Standard in the dark.

Application: For detection and quantification of Acid Phosphatase activity in serum, plasma, and tissue homogenates.

Introduction

Acid Phosphatase (ACP) is an enzyme found in animal and plant cells which removes phosphate groups ($R\text{-}O\text{-}PO_3^{2-}$) to produce free phosphate, leaving behind a hydroxyl group (R-OH). In cells, it is typically found within the highly acidic lysosomes, assisting in their primary role of breaking down large molecules into smaller, more useful products. As such, Acid Phosphatase operates well at a low pH. Its prevalence in cells and serum has historically made Acid Phosphatase a useful biomarker for researchers, including in the discovery of the lysosome itself, and as an indicator of prostate cancer.

Abbexa's Acid Phosphatase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Acid Phosphatase activity. Acid Phosphatase hydrolyzes disodium p-nitrophenyl phosphate (pNPP) to produce p-nitrophenol, a yellow compound with an absorbance maximum of 405 nm. The intensity of the color is proportional to the Acid Phosphatase activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Assay Buffer: 20 ml
3. Chromogenic Reagent: 24 ml
4. Substrate: 3 vials
5. Standard: 1 vial
6. Plate sealer: 2

Materials required but not provided

1. Microplate reader (405 nm)
2. Distilled water
3. PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. 1.5 ml microcentrifuge tubes
6. Centrifuge
7. Vortex mixer
8. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum** and **Plasma:** Serum and plasma samples can be tested directly.
- **Tissue Homogenates:** Carefully weigh up to 1 g of tissue, and add into PBS (0.01 M, pH 7.4) in a ratio of 1:9 mass (g) to volume (ml) (i.e. for 1 g of tissue, add 9 ml of PBS). Homogenize manually, using a mechanical homogenizer or by ultrasonication, in an ice water bath. Centrifuge at 10,000 × g for 10 minutes at 4°C, then collect the supernatant for and assay immediately.

  For tissue homogenate analysis: Determine the total protein concentration in the supernatant. The total protein concentration will be used in **C. Calculation of Results**.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with PBS (0.01 M, pH 7.4), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% *Epipremnum aurem* tissue homogenate	5 – 10
Mouse plasma	5 – 10
Rat plasma	5 – 10
Human plasma	5 – 10
Human urine	1
10% Rat spleen tissue homogenate	20 – 30
10% Rat liver tissue homogenate	20 – 30
10% Rat kidney tissue homogenate	20 – 30

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Substrate Working Solution**: Dissolve the Substrate with 1.6 ml Assay Buffer. Use immediately, or store the Substrate Working Solution for up to 24 hours at -20°C in the dark.
- **10 mmol/L Standard Solution:** Reconstitute the lyophilized Standard with 5 ml distilled water and mix fully to prepare a 10 mmol/L Standard Solution. It is recommended to aliquot this solution, and store any unused reagent for up to 7 days at -20°C in the dark.
- **0.5 mmol/L Standard Solution:** Dilute the 10 mmol/L Standard Solution with Assay Buffer in a ratio of 1:19. Prepare this solution just before use, and keep in the dark until just before use.
- **Standard Dilutions:** Label 7 tubes with 0.5 mmol/L, 0.4 mmol/L, 0.3 mmol/L, 0.25 mmol/L, 0.2 mmol/L, 0.1 mmol/L, and 0.05 mmol/L. Add 200 µl, 160 µl, 120 µl, 100 µl, 60 µl, 40 µl, and 20 µl of 0.5 mmol/L Standard Solution to the 0.5 mmol/L, 0.4 mmol/L, 0.3 mmol/L, 0.25 mmol/L, 0.2 mmol/L, 0.1 mmol/L, and 0.05 mmol/L tubes respectively, followed by 0 µl, 40 µl, 80 µl, 100 µl, 120 µl, 160 µl, and 180 µl of Assay Buffer, to prepare Standard Dilutions with concentrations 0.5 mmol/L, 0.4 mmol/L, 0.3 mmol/L, 0.25 mmol/L, 0.2 mmol/L, 0.1 mmol/L, and 0.05 mmol/L. These volumes are summarized in the following table:

Standard Dilution (mmol/L)	**0.5**	**0.4**	**0.3**	**0.25**	**0.2**	**0.1**	**0.05**
0.5 mmol/L Standard (µl)	200	160	120	100	80	40	20
Assay Buffer (µl)	0	40	80	100	120	160	180

For the blank, or 0 mmol/L Standard, use pure Assay Buffer. The volume of each standard will be 200 µl. In total, there will be 8 standard dilutions (0.5 mmol/L, 0.4 mmol/L, 0.3 mmol/L, 0.25 mmol/L, 0.2 mmol/L, 0.1 mmol/L, 0.05 mmol/L, and blank).

Note:

- Allow all reagents to equilibrate to room temperature before use.
- Keep the Substrate Working Solution in the dark, and dispose of any unused Substrate Working Solution at the end of the assay (do not store or re-use).

B. Assay Procedure

1. Mark positions for each standard, sample, and control. Each sample requires a corresponding control. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 40 µl of each standard dilution into the corresponding standard wells.
3. Add 40 µl of sample into each sample well.
4. Add 40 µl of sample into the corresponding control wells.
5. Add 40 µl of Assay Buffer into the standard wells and control wells.
6. Add 40 µl of Substrate Working Solution into the sample wells.
7. Shake the microplate to mix fully, and then incubate at 37°C for 10 minutes.
8. Add 160 µl of Chromogenic Reagent to all wells.
9. Shake the microplate to mix fully for 3 seconds, and then measure the OD of each well with a microplate reader at 405 nm.

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of Acid Phosphatase in each sample well can be derived with the following formulae:

1. Serum and Plasma samples:

One unit of Acid Phosphatase activity is defined as the amount required for 1 L of serum or plasma to produce 1 µmol of p-nitrophenol per minute at 37°C.

$$\text{Acid Phosphatase (U/L)} = \mathrm{F} \times 1000 \times \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Control}} - \mathrm{b})}{\mathrm{a} \times \mathrm{t}}$$

2. Tissue samples:

One unit of Acid Phosphatase activity is defined as the amount required for 1 g of tissue protein to produce 1 µmol of p-nitrophenol per minute at 37°C.

$$\text{Acid Phosphatase (U/L)} = \mathrm{F} \times 1000 \times \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Control}} - \mathrm{b})}{\mathrm{a} \times \mathrm{t} \times \mathrm{C_{Protein}}}$$

where:

OD_{Sample}	OD value of sample
$OD_{Control}$	OD value of control
$C_{Protein}$	Concentration of total protein in sample (g/L)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
t	Time of the reaction (10 mins)
F	The dilution factor of sample