Acid Phosphatase (ACP) Assay Kit

Catalog No.: abx294068

Size: 96 tests

Detection Range: 14 U/L – 400 U/L

Sensitivity: 14 U/L

Storage: Store all components at 4°C in the dark.

Application: For detection and quantification of Acid Phosphatase (ACP) activity in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, urine and hydrothorax.

Principle of the Assay

Abbexa's Acid Phosphatase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Acid Phosphatase activity. Under acidic conditions, ACP breaks down disodium phenyl phosphate to produce free phenol and phosphoric acid. Under alkaline conditions and catalyzed by potassium ferricyanide, the free phenol reacts with 4-aminoantipyrine to form a red quinone derivative, with an absorbance maximum at 520 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. Substrate Solution: 2 ml
3. Buffer Solution: 2 ml
4. Chromogenic Reagent: 2 × 9 ml
5. Alkaline Reagent: 2 × 6 ml
6. Standard (1 mg/ml): 2 ml
7. Plate sealer: 2

Materials required but not provided

1. Microplate reader (520 nm)
2. Double-distilled water
3. Normal saline (0.9 % NaCl)
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. 1.5 ml microcentrifuge tubes
7. Centrifuge
8. Vortex mixer
9. Mechanical homogenizer
10. Ultrasonicator
11. Incubator

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Protocol

A. Preparation of samples and reagents

1. Reagents

- **Substrate Working Solution:** Prepare enough Substrate Working Solution for the wells to be tested. Mix Substrate Solution and Buffer Solution in a ratio of 1 : 1. For example, prepare 25 µl of Substrate Working Solution by mixing 12.5 µl of Substrate Solution with 12.5 µl of Buffer Solution. Prepare the Substrate Working Solution immediately before use. Store at 4°C and use within 24 hours.
- **Standards:** Label 7 tubes with 1 mg/ml, 0.8 mg/ml, 0.6 mg/ml, 0.5 mg/ml, 0.4 mg/ml, 0.2 mg/ml, and 0.1 mg/ml. Prepare these dilutions according to the volumes in the following table:

Standard Dilution (mg/ml)	1	0.8	0.6	0.5	0.4	0.2	0.1
1 mg/ml Standard (µl)	200	160	120	100	80	40	20
Double-distilled water (µl)	0	40	80	100	120	160	180

For the blank, or 0 mg/ml standard, use pure double-distilled water. The volume of each standard will be 200 µl.

Note: Allow all reagents to equilibrate to room temperature before use.

2. 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 at least 20 mg of tissue and wash the tissue with cold PBS (0.01 M, pH 7.4). Per 20 mg of tissue, add 180 µl normal saline (0.9 % NaCl) or PBS (0.01 M, pH 7.4). Homogenize manually or use a mechanical homogenizer at 4°C. Centrifuge at 10,000 × g for 10 minutes at 4°C. Carefully collect the supernatant and keep it on ice for detection.
- **Cell Samples (Adherent or Suspension Cultures):** Collect at least 1 × 10^6 cells and wash the cells with PBS (0.01 M, pH 7.4). Per 1×10^6 cells, add 300 µl normal saline (0.9 % NaCl) or PBS (0.01 M, pH 7.4). Sonicate at 4 °C for homogenization. Centrifuge at 10,000 × g for 10 minutes at 4°C. Carefully collect the supernatant and keep on ice for detection.

Note: To calculate Acid Phosphatase activity in tissue homogenates or cell samples using the formulae in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately **(abx 097193)**.

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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 normal saline (0.9 % NaCl) or 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
Human serum	1
Human saliva	1
Chicken serum	1
Human urine	1
10 % Rat liver tissue homogenates	4 – 10
10 % Rat kidney tissue homogenates	4 – 10
Hep G2 Cells	1

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.

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B. Assay Procedure

1. Mark positions on the 96-well microplate for each standard, blank and sample. *It is strongly recommended to test all wells in duplicate*.
2. Add 5 µl of sample to each sample well.
3. Add 5 µl of double-distilled water to the blank well.
4. Add 5 µl of the prepared standard dilutions to the corresponding standard wells.
5. Add 25 µl of Substrate Working Solution to all wells.
6. Mix the well contents by tapping the plate, or shaking with a microplate shaker for approximately 10 seconds.
7. Cover with a plate sealer and incubate at 37°C for 30 minutes.
8. Unseal the plate, then add 100 µl of Alkaline Reagent and 150 µl of Chromogenic Reagent to all wells.
9. Mix the well contents by tapping the plate, or shaking with a microplate shaker for approximately 10 seconds.
10. Cover with a plate sealer and incubate at room temperature for 5 minutes.
11. Unseal the plate and measure the OD of each well with a microplate reader at 520 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 activity 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 of enzyme in 100 ml of serum or plasma required to produce 1 mg of phenol per 30 minutes at 37°C.

$$\text{Acid Phosphatase activity (U/ 100 ml)} = \mathrm{F} \times \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Blank}} - \mathrm{b}) \times \mathrm{V}}{\mathrm{a}}$$

2. Tissue and cell samples:

One unit of Acid Phosphatase activity is defined as the amount of enzyme in 1 g of tissue protein required to produce 1 mg of phenol per 30 minutes at 37°C.

$$\text{Acid Phosphatase activity (U/g Protein)} = \mathrm{F} \times \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Blank}} - \mathrm{b})}{\mathrm{a} \times \mathrm{C_{Protein}}}$$

where:

$\mathrm{OD_{Sample}}$	OD value of sample
$\mathrm{OD_{Blank}}$	OD value of blank standard
V	Defined volume of sample (100 ml)
$\mathrm{C_{Protein}}$	Concentration of protein in sample (g Protein/ml)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
F	The dilution factor of sample

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Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.