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CheKine™ Mirco Acid Phosphatase (ACP) Activity Assay Kit

Cat #: KTB2360 Size: 48 T/24 S 96 T/48 S

	Mirco Acid Phosphatase (ACP) Activity Assay Kit		
REF	**Cat #:** KTB2360	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant Tissues, Serum (Plasma)		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

ACP (Acid Phosphatase) catalyzes the hydrolysis of phosphomonoesters into inorganic phosphate under acidic conditions, commonly found within lysosomes of macrophages. ACP is frequently employed as an auxiliary diagnostic tool for prostate cancer. CheKine™ Micro Acid Phosphatase (ACP) Activity Assay Kit provides a simple, convenient, and rapid method for determining ACP activity, applicable to a variety of samples including plant and animal tissues, serum, and plasma. Its principle relies on the ACP-catalyzed hydrolysis of sodium phenyl phosphate to produce phenol in an acidic milieu; subsequently, phenol reacts with 4-aminoantipyrene and potassium ferricyanide to form a red quinone derivative which exhibits characteristic light absorption at 510 nm. By measuring the rate of increase in absorbance at 510 nm, the ACP activity can be calculated.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	120 mL	4℃
Reagent Ⅰ	3 mL	6 mL	4℃, protected from light
Reagent Ⅱ	3 mL	6 mL	4℃, protected from light
Reagent Ⅲ	9 mL	18 mL	4℃, protected from light
Standard	0.5 mL	0.5 mL	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 510 nm
- Incubator, ice maker, freezing centrifuge
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Deionized water
- Dounce homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
Reagent II: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
Standard: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
Note: Reagent III is toxic and Extraction Buffer and Standard have a pungent odor, so it is recommended to experiment in a fume hood.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Animal and tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 10,000 rpm for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Serum or Plasma: Plasma and serum can be directly measured. EDTA and citrate cannot be used in plasma preparation, andother anticoagulants can be used.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 510 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Preheat Reagent Ⅰ in a 37℃ water bath for more than 30 min.
3. Operation table (The following operations are operated in the 96-well plate):

Reagent	Test Well (μL)	Control Well (μL)	Blank Well (μL)	Standard Well (μL)
Deionized Water	0	0	20	0
Standard	0	0	0	20
Sample	20	0	0	0
Reagent Ⅰ	40	40	40	40
Reagent Ⅱ	40	40	40	40
Mix and place in 37℃ incubation for 15 min				
Reagent Ⅲ	120	120	120	120
Sample	0	20	0	0
Mix and measure absorbance at 510 nm, it only needs one standard well and blank well. Every sample needs to set a control well. The absorbance of each tube recorded as A_{Test}, $A_{Control}$, A_{Blank}, $A_{Standard}$, respectively. Calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.				

Note: Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment. If ΔA_{Test} is less than 0.1, the sample volume can be appropriately increased. If ΔA_{Test} is greater than 1.5, the sample can be further diluted with Extraction Buffer before proceeding with the experiment, and the final dilution factor should be taken into account in the calculations.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Calculated by protein concentration

Active unit definition: At 37℃,1 μmol phenol produced per min in 1 mg protein reaction system is defined as a unit of enzyme activity.

ACP (U/mg prot)=($C_{Standard}$×ΔA_{Test}÷$\Delta A_{Standard}$×V_{Sample})÷(Cpr×V_{Sample})÷T=**ΔA_{Test}÷$\Delta A_{Standard}$÷15÷Cpr**

2. Calculated by sample fresh weight

Active unit definition: At 37℃,1 μmol phenol produced per min in 1 g tissue reaction system is defined as a unit of enzyme activity.

ACP (U/g fresh weight)=($C_{Standard}$×ΔA_{Test}÷$\Delta A_{Standard}$×V_{Sample})÷(W÷$V_{Extract}$×V_{Sample})÷T=**ΔA_{Test}÷$\Delta A_{Standard}$÷15÷W**

3. Calculated by solution volume

Active unit definition: At 37℃,1 μmol phenol produced per min in 1 mL plasma or serum reaction system is defined as a unit ofenzyme activity.

ACP (U/mL)=($C_{Standard}$×ΔA_{Test}÷$\Delta A_{Standard}$×V_{Sample})÷V_{Sample}÷T=**ΔA_{Test}÷$\Delta A_{Standard}$÷15**

Where: $C_{Standard}$: Concentration of Standard; 2 μmol/mL; V_{Sample}: Supernatant volume added to the reaction system, 0.02 mL; T: Reaction time, 15 min; $V_{Extract}$: Extraction Buffer added, 1 mL; W: Sample fresh weight, g; Cpr: Supernatant protein concentration,mg/mL.

Precautions

1. Reagent Ⅰ, Reagent Ⅱ, and Reagent Ⅲ all require storage protected from light.
2. Reagent Ⅲ cannot be used once it turns bluish-green.
3. After adding Reagent Ⅲ, immediate mixing is essential; otherwise, incomplete color development may occur.
4. ACP is unstable, particularly at 37℃ and pH levels above 7, leading to rapid loss of activity. Therefore, ACP samples are typically prepared fresh daily. In serum samples, to lower the pH below 6.5, either 10 mg of disodium hydrogen citrate or 5 mg of sodium bisulfate should be added per milliliter of serum. Alternatively, for 5 mL of serum, 2 to 3 drops of 30% acetic acid solution can be added, and the sample can be stored at 4℃ for up to one week.

Typical Data

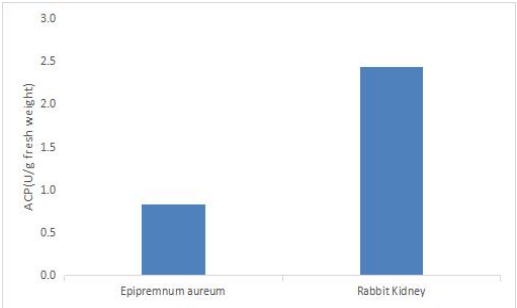

Figure 1. Determination ACP activity in Epipremnum aureum and Rabbit kidney by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-Glucosidase Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.