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CheKine™ Micro Alkaline Proteinase (AKP) Activity Assay Kit

Cat #: KTB2280 Size: 48 T/96 T

	Micro Alkaline Proteinase (AKP) Activity Assay Kit		
REF	**Cat #:** KTB2280	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal Tissues, Bacteria or Fungus, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Alkaline proteinase (AKP) refers to enzymes that catalyze the hydrolysis of protein peptide bonds under alkaline conditions, belonging to serine proteinase. In addition, the enzyme can also hydrolyze ester bonds and amide bonds, and has the function of converting esters and peptides. This enzyme is one of the main industrial enzymes widely used in industries such as pharmaceuticals, silk, food, and leather. Under alkaline conditions, AKP hydrolyzes casein to produce tyrosine, which reduces phosphomolybdic acid to produce tungsten blue; Tungsten blue has a characteristic absorption peak at 680 nm, and the rate of absorbance increase at 680 nm is measured to calculate AKP activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	1	1	4℃, protected from light
Reagent Ⅱ	1	1	4℃, protected from light
Reagent Ⅲ	1	1	4℃
Reagent Ⅳ	2.5 mL	5 mL	4℃, protected from light
Standard	1 mL	1 mL	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 680 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL eppendorf tube
- Water bath,incubator, ice maker, centrifuge, magnetic stirrer
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Reagent I: Prepared before use. 48 T add 2.5 mL deionized water, 96 T add 5 mL deionized water to fully dissolve; Unused reagents can be stored in the dark at 4℃ for one week.

Reagent II: Prepared before use. 48 T add 5 mL Reagent I, 96 T add 10 mL Reagent I, and dissolve by magnetic stirring in boiling water bath. (You can cover the beaker with a layer of plastic/cling wrap, pay attention to observation, avoid all evaporation of water, generally heat for 15-30 min, the reagent is supersaturated, and the use of insoluble particles will not be affected after full mixing), Unused reagents can be stored in the dark at 4℃ for one week.

Reagent III: Prepared before use. 48 T add 10 mL deionized water, 96 T add 20 mL deionized water to fully dissolve; Unused reagents can be stored at 4℃ for one month.

Reagent IV: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

Stardard： Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

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 tissues: Weigh 0.1 g tissue, add 1 mL Reagent I and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, that is the crude enzyme solution, and place it on ice to be tested.
2. Bacteria or Fungus: Collect 5×10^6 bacteria or fungus into the centrifuge tube, wash fungus or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Reagent I to ultrasonically disrupt the bacteria or fungus 3 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, that is the crude enzyme solution, and place it on ice to be tested.
3. Plasma, Serum or other Liquid samples: Direct detection.

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 680 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Incubate Reagent I、II、III at 40℃ for 30 min.
3. Sample measurement. (The following operations are operated in the 1.5 mL eppendorf tube)

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)	Control Tube (μL)
Crude Enzyme Solution	0	0	20	20
Reagent I			0	40
Reagent II			40	0
			Mix thoroughly, incubate at 40℃ for 30 min	
Reagent I			40	0
Reagent II			0	40
			After mixing, centrifuge at 8,000 g for 10 min at 4℃, take the supernatant, add the following reagents to the new EP tube	

Supernatant	0	0	40	40
Standard	0	40	0	0
Deionized Water	40	0	0	0
Reagent III	200	200	200	200
Reagent IV	40	40	40	40
Mix thoroughly, put in 40℃ water bath for 20 min				

4. Add 200 μL to micro glass cuvette or 96 well plate, detect the absorbance at 680 nm. The absorbance of test tube, control tube, standard tube and blank tube were recorded as A_{Test}, $A_{Control}$, $A_{Standard}$ and A_{Blank}. Calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Each test well needs to be equipped with a control well, standard curve and blank well only need to be done once or twice. Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment. If reaction is weak and ΔA_{Test} is small, prolong the water bath time of the first step (20-30 min), and the formula should be modified when calculating the enzyme activity.

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.

Calculation of the AKP activity

(1) Calculated by sample protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μmol of tyrosine in the reaction system per min at 40℃ every mg protein.

$AKP(U/mg\ prot)=C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Total\ volume}\div(Cpr\times V_{Reation})\div T=\mathbf{0.125\times\Delta A_{Test}\div\Delta A_{Standard}\div Cpr}$

(2) Calculated by fresh weight of samples

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μmol of tyrosine in the reaction system per min at 40℃ every g sample.

$AKP(U/g\ fresh\ weight)=C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Total\ volume}\div(W\times V_{Reation}\div V_{Extraction})\div T=\mathbf{0.125\times\Delta A_{Test}\div\Delta A_{Standard}\div W}$

(3) Calculated by bacteria or fungus

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μmol of tyrosine in the reaction system per min at 40℃ every 10^4 bacteria or fungus.

$AKP(U/10^4)=C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Total\ volume}\div(n\times V_{Reation}\div V_{Extraction})\div T=\mathbf{0.125\times\Delta A_{Test}\div\Delta A_{Standard}\div n}$

(4) Calculated by volume of liquid samples

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μmol of tyrosine in the reaction system per min at 40℃ every mL sample.

$AKP(U/mL)=C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Total\ volume}\div V_{Reation}\div T=\mathbf{0.125\times\Delta A_{Test}\div\Delta A_{Standard}}$

$C_{Standard}$: Standard tyrosine solution, 0.25 μmol/mL; $V_{Total\ volume}$: Reaction total volume, 0.1 mL; Cpr: Sample protein concentration, mg/mL; $V_{Reation}$: The volume of crude enzyme was added to the reaction system, 0.02 mL; $V_{Extraction}$: Total volume of Extraction Buffer, 1 mL; T: The reaction time, 10 min; W: Sample weight, g; n: Bacteria or fungus amount.

Typical Data

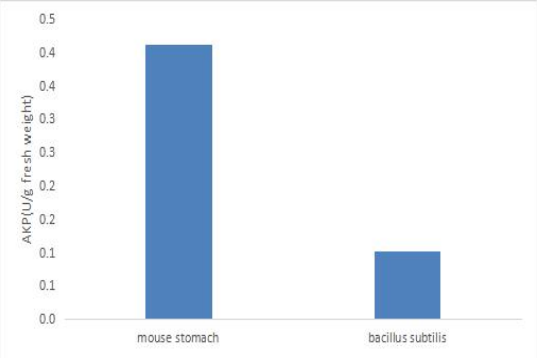

Figure 1. Determination of AKP activity in mouse stomach and bacillus subtilis by this kit.

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-Glucosidase Activity Assay Kit
KTB1121	CheKine™ Pyruvate Acid (PA) Colorimetric Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.