CheKine™ Micro Acidic Proteinase (ACP) Activity Assay Kit

Cat #: KTB2270 Size: 48 T/96 T

	Micro Acidic Proteinase (ACP) Activity Assay Kit		
REF	**Cat #:** KTB2270	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

Acidic proteinase is an enzyme that catalyzes the hydrolysis of proteins in acidic environments. The enzyme is mainly used in alcohol fermentation, beer brewing, fur softening, fruit wine clarification, soy sauce brewing, feed and so on. CheKine™ Micro Acidic Proteinase (ACP) Activity Assay Kit can be used to detect biological samples such as animal tissues, bacteria or fungus, serum or plasma. In the kit, in acidic condition, ACP can catalyze the hydrolysis of casein to produce tyrosine; in alkaline condition, tyrosine reduces phosphomolybdic acid compound to tungsten blue which has characteristic absorption peak at 680 nm, and the activity of ACP is calculated by measuring its absorbance increase.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Liquid Ⅰ	1 mL	2 mL	4℃, protected from light
Liquid Ⅱ	0.5 mL	1 mL	4℃, protected from light
Reagent Ⅱ	1	1	4℃, protected from light
Reagent Ⅲ	1	1	4℃, protected from light
Reagent Ⅳ	1	1	4℃
Reagent Ⅴ	2 mL	4 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 pot, cryogenic centrifuge machine
- Deionized water

• Homogenizer (for tissue samples)

Reagent Preparation

Reagent I: Prepared before use. According to the ratio of Liquid Ⅰ: Liquid II: deionized water=90(μL): 20(μL): 21(mL). add 2 mL deionized water to fully dissolve. The prepared reagent can be stored at 4℃, protected from light for 4 weeks.

Reagent II: Prepared before use. 48 T add 2 mL deionized water, 96 T add 4 mL deionized water to fully dissolve; Store at 4℃, protected from light.

Reagent Ⅲ: 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 fresh-keeping film, pay attention to observation, avoid all evaporation of water, generally heat for 15-30 minutes, the reagent is supersaturated, and the use of insoluble particles will not be affected after full mixing.)Store at 4℃, protected from light.

Reagent Ⅳ: Prepared before use. 48 T add 10 mL deionized water, 96 T add 20 mL deionized water to fully dissolve; Store at 4℃.

Reagent Ⅴ: 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 12,000 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.

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. Reagent Ⅱ、Ⅲ、Ⅳ were kept in 30℃ water bath for 30 min.
3. Sample measurement. (The following operations are operated in the 1.5 mL eppendorf tube)

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)	Control Well (μL)
Sample	0	0	20	20
Reagent Ⅱ			0	40
Reagent Ⅲ			40	0
			Mix thoroughly, put in 30℃ water bath for 10 minutes	
Reagent Ⅱ			40	0
Reagent Ⅲ			0	40
			After mixing, centrifuge at 8,000 g for 10 min at 4℃, take the supernatant	

Supernatant	0	0	40	40
Standard	0	40	0	0
Deionized water	40	0	0	0
Reagent Ⅳ	200	200	200	200
Reagent Ⅴ	40	40	40	40
Mix thoroughly, put in 30℃ water bath for 20 minutes				

4. Add 200 μL to micro glass cuvette/96 well flat-bottom plate, detect the absorbance at 680 nm after cooling with running water. The Blank Well is recorded as A_{Blank}, the Standard Well is marked as $A_{Standard}$, the Test Well is marked as $A_{Test,}$ and the Control Well is marked as $A_{Control.}$ Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note:The Blank Well and the Standard Well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.001, increase the sample quantity appropriately. If ΔA is greater than 2, the sample can be appropriately diluted with deionized water, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

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 ACP 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 minute at 30℃ every mg protein.

$ACP(U/mg\ prot)= C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Total\ volume}\div(Cpr\times V_{Reation})\div T$**$=125\times\Delta A_{Test}\div\Delta A_{Standard}\div Cpr$**

(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 minute at 30℃ every g sample.

$ACP(U/g\ fresh\ weight)=C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Total\ volume}\div(W\times V_{Reation}\div V_{Extraction})\div T$**$=125\times\Delta A_{Test}\div\Delta A_{Standard}\div W$**

(3) Calculated by bacteria or 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 minute at 30℃ every 10^4 bacteria or fungus.

$ACP(U/10^4\ bacteria\ or\ fungus)=C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Total\ volume}\div(n\times V_{Reation}\div V_{Extraction})\div T$**$=125\times\Delta A_{Test}\div\Delta A_{Standard}\div n$**

(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 minute at 30℃ every mL sample.

$ACP(U/mL)=C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Total\ volume}\div V_{Reation}\div T$**$=125\times\Delta A_{Test}\div\Delta A_{Standard}$**

$C_{Standard}$: 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, 0.04 mL; $V_{Extraction}$: Total volume of extractive liquid; T: The reaction time, 10 min; W: Sample weight, g; n: Bacteria or fungus amount.

Precautions

1. Reagent Ⅰ is prepared according to the operation instructions and is now in use. if there is a white flocculent precipitation, it cannot be used.
2. The reagents prepared before use will be used within 3 days after configuration.
3. If reaction is weak and (A_{Test}-$A_{Control}$) 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.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

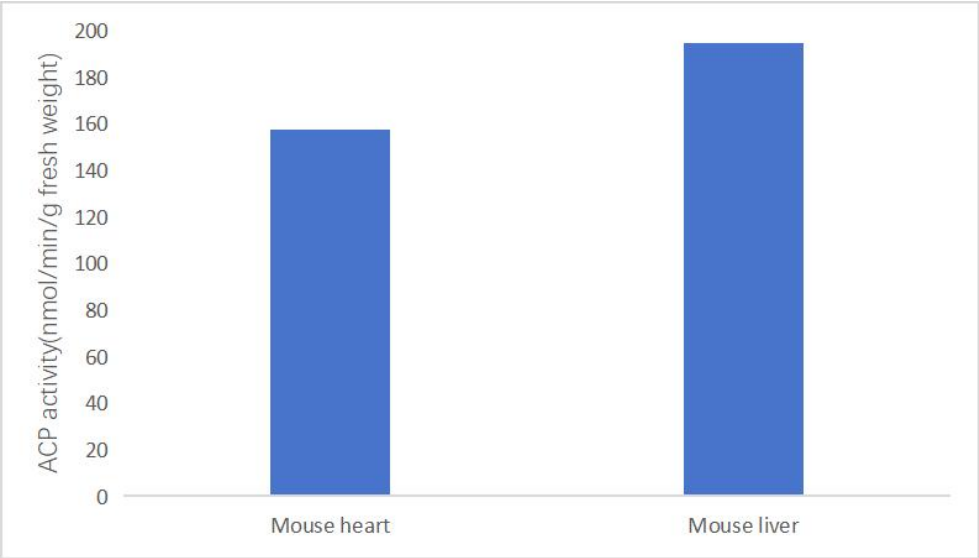

Figure 1. Determination of ACP activity in mouse heart and liver by this kit.

Recommended Products

Catalog No.	Product Name
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit
KTB1040	CheKine™ Micro Catalase (CAT) Activity Assay Kit
KTB1110	CheKine™ Lactate Dehydrogenase (LDH) Activity Assay Kit
KTB1640	CheKine™ Micro Glutathione Peroxidase (GSH-Px) Activity Assay Kit

Disclaimer

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