CheKine™ Micro Pepsase Activity Assay Kit

Cat #: KTB2340 Size: 48 T/24 S 96 T/48 S

	Micro Pepsase Activity Assay Kit		
REF	**Cat #:** KTB2340	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal Tissues		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Pepsin is secreted by the main cells of the gastric mucosa and breaks down proteins in food into small peptides. Generally used for the identification of neuropathic hypoacidosis, chronic gastritis, chronic gastric dilatation, chronic duodenitis and other symptoms will also cause the decrease of pepsin secretion. CheKine™ Micro Pepsase Activity Assay Kit can be used to detect biological samples such as animal tissues. In the kit, pepsase can catalyze the hydrolysis of hemoglobin, and the hydrolysate appears blue after reacting with Folin reagent, and the color of pepsin is proportional to the activity of pepsin in a certain range.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	60 mL	120 mL	4℃
Reagent Ⅱ	10 mL	20 mL	4℃
Reagent Ⅲ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅳ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅴ	Powder×1 vial	Powder×1 vial	4℃
Reagent Ⅵ	1.66 mL	3.3 mL	4℃, protected from light
Standard	1.27 mL	1.27 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 580 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath, cryogenic centrifuge
- Deionized water

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• Homogenizer or mortar

Reagent Preparation

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

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

Working Reagent III: Prepared before use. 48 T add 6 mL Reagent II, 96 T add 12 mL Reagent II, fully dissolve. Inexhaustible reagents stored at 4℃ for 1 week, protected from light.

Working Reagent IV: Prepared before use. 48 T add 6 mL deionized water, 96 T add 12 mL deionized water, fully dissolve. Inexhaustible reagents stored at 4℃ for 1 week, protected from light.

Working Reagent V: Prepared before use. 48 T add 7.5 mL deionized water, 96 T add 15 mL deionized water, fully dissolve. Inexhaustible reagents stored at 4℃ for 1 week.

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

Note: Reagent IV, Reagent V or Reagent VI has certain irritation, so personal protection is recommended during use.

Standard： 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.

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, and place it on ice to be tested.

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 580 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Preheat Reagent III, Reagent IV at 37℃ for 30 min.
3. Sample measurement. (The following operations are operated in the 1.5 mL EP tube)

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)	Control Well (μL)
Sample	0	0	20	0
Deionized Water	0	0	0	100
Working Reagent III	0	0	100	0
Heat preservation 10 min in 37℃ water bath.				
Working Reagent IV	0	0	100	100
Cover tightly and shake 1 min well.				
Sample	0	0	0	20
After mixing, centrifuged at 8,000 g for 10 min at 4℃ to extract the supernatant. The following operations are operated in the 96-well plate or microglass cuvette				
Supernatant	0	0	20	20
Standard	0	20	0	0

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Deionized Water	20	0	0	0
Reagent II	40	40	40	40
Working Reagent V	120	120	120	120
Reagent VI	20	20	20	20

4. Mix well and let stand at room temperature for 20 min, detect the absorbance at 580 nm. 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_{Test} is less than 0.002, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.6, 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 pepsase activity

(1) Calculated by sample protein concentration

Unit definition: One unit of enzyme is defined as the catalytic hydrolysis of hemoglobin to 1 nmol tyrosine per milligram of protein at 37℃.

Pepsase (U/mg prot)=$C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Sample}\times F\div(Cpr\times V_{Sample})\div T$**=$550\times\Delta A_{Test}\div\Delta A_{Standard}\div Cpr$**

(2) Calculated by fresh weight of samples

Unit definition: One unit of enzyme is defined as the catalytic hydrolysis of hemoglobin to 1 nmol tyrosine per gram tissue weight at 37℃.

Pepsase (U/g fresh weight)=$C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Sample}\times F\div(W\times V_{Sample}\div V_{Total\ sample})\div T$**=$550\times\Delta A_{Test}\div\Delta A_{Standard}\div W$**

$C_{Standard}$: Concentration of standard solution, 0.5 μmol/mL; F: Dilution multiple, (20+100+100)÷20=11; $V_{Total\ sample}$: The volume of Sample, 1 mL; V_{Sample}: Added sample volume to the reaction system, 0.02 mL; Cpr: Sample protein concentration, mg/mL; T: Reaction time, 10 min; W: Sample weight, g.

Typical Data

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

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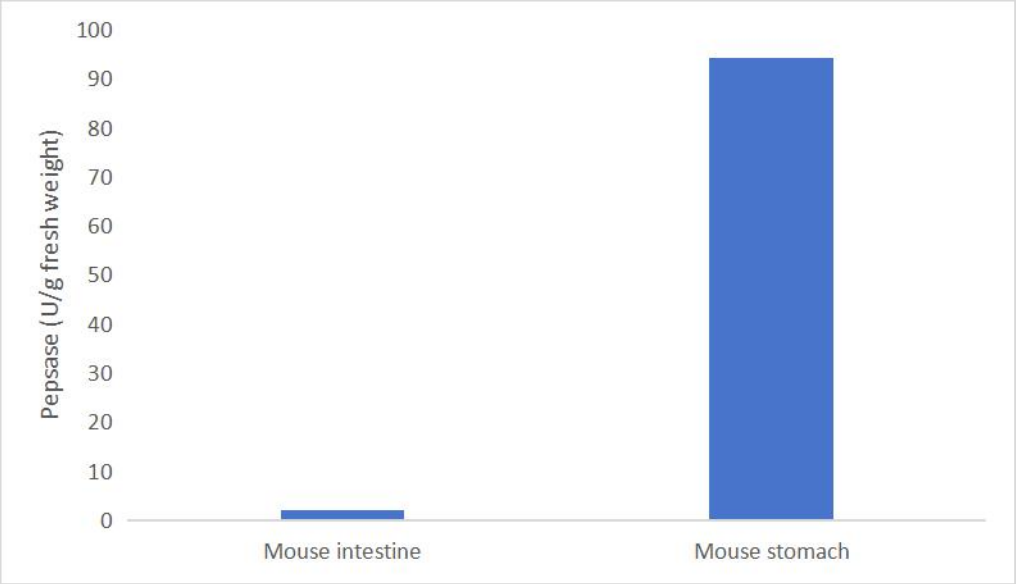

Figure 1. Determination of pepsase activity in mouse intestine and stomach by this kit.

Recommended Products

Catalog No.	Product Name
KTB2270	CheKine™ Micro Acid Protease (ACP) Activity Assay Kit
KTB2280	CheKine™ Micro Alkaline protease (AKP) Activity Assay Kit
KTB2290	CheKine™ Micro Alkaline protease (AKP) 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.

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