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CheKine™ Micro Trypsase Activity Assay Kit

Cat #: KTB2320 Size: 48 T/96 T

	Micro Trypsase Activity Assay Kit		
REF	**Cat #:** KTB2320	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Trypsase is a serine protease from the PA clan superfamily, found in the digestive system of many vertebrates, where it hydrolyses proteins. In the duodenum, trypsase catalyzes the hydrolysis of peptide bonds, breaking down proteins into smaller peptides. The peptide products are then further hydrolyzed into amino acids via other proteases, rendering them available for absorption into the blood stream. Trypsase is widely used in the treatment of local edema, hematoma and abscess due to the pyothorax, hemothorax, surgical inflammation, ulcer, traumatic injury, etc. CheKine™ Micro Trypsase Activity Assay Kit can be used to detect biological samples such as animal and plant tissues. In the kit, the ester bond of TAME was hydrolyzed by trypsase, and the free carboxyl released neutralized with sodium hydroxide in the reaction system, resulting in the decrease of pH value of the solution. The trypsase activity data can be quickly measured by using phenol red as an indicator to determine the change of the absorption value of the solution at 555 nm. There was a good linear relationship between trypsase and the decrease of absorption value at 555 nm in a certain range.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	2.5 mL	5 mL	4℃, protected from light
Reagent Ⅱ	2.5 mL	5 mL	4℃, protected from light
Reagent Ⅲ	1 mL	2 mL	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 555 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath, cryogenic centrifuge
- Deionized water

• Homogenizer or mortar (for tissue samples)

Reagent Preparation

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

Working Reagent: Prepared before use. According to the dosage, it is fully mixed according to the ratio of Reagent Ⅰ (V): Reagent Ⅱ(V): deionized water(V): Reagent Ⅲ(V)=1 mL: 1 mL: 5.4 mL: 0.4 mL, This reagent is worthy of now being off-the-shelf. **(Note: Mix as much as you want, prepare in an empty bottle, with 2 empty bottles in 48 T and 4 empty bottles in 96 T)**

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.

Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant, that is crude enzyme solution, 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 555 nm. Visible spectrophotometer was returned to zero with deionized water.

2. Sample measurement. (The following operations are operated in the 96-well plate or microglass cuvette)

Reagent	Test Well (μL)
Sample	5
Working Reagent	195

3. Determine immediately after mixing, detect the absorbance at 555 nm as A1 after 1 min and A2 after 2 min. Finally calculate ΔA=A2-A1.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.005, reaction time can be extended to 5 min. If ΔA is greater than 0.5, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor.

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 trypsase activity

(1) Calculated by sample protein concentration

Unit definition: At room temperature, the absorbance at the catalytic 555 nm per min per milligram of protein decreased by 0.5 to 1 unit of enzyme activity.

Trypsase (U/mg prot)=ΔA×$V_{Total\ volume}$÷(Cpr×V1)÷0.5÷T**=80×ΔA÷Cpr**

(2) Calculated by fresh weight of samples

Unit definition: At room temperature, the absorbance at the catalytic 555 nm per min per gram tissue decreased by 0.5 to 1 unit of enzyme activity.

Trypsase (U/g fresh weight)=ΔA×$V_{Total\ volume}$÷(W×V1÷V2)÷0.5÷T**=80×ΔA÷W**

Cpr: Sample protein concentration, mg/mL; W: Sample weight, g; V1: The volume of crude enzyme was added to the reaction

system, 5 μL=0.005 mL; V2: Total volume of crude enzyme solution, 1 mL; $V_{Total\ volume}$: Total volume of reaction, 0.2 mL; T: Reaction time, 1 min.

Typical Data

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

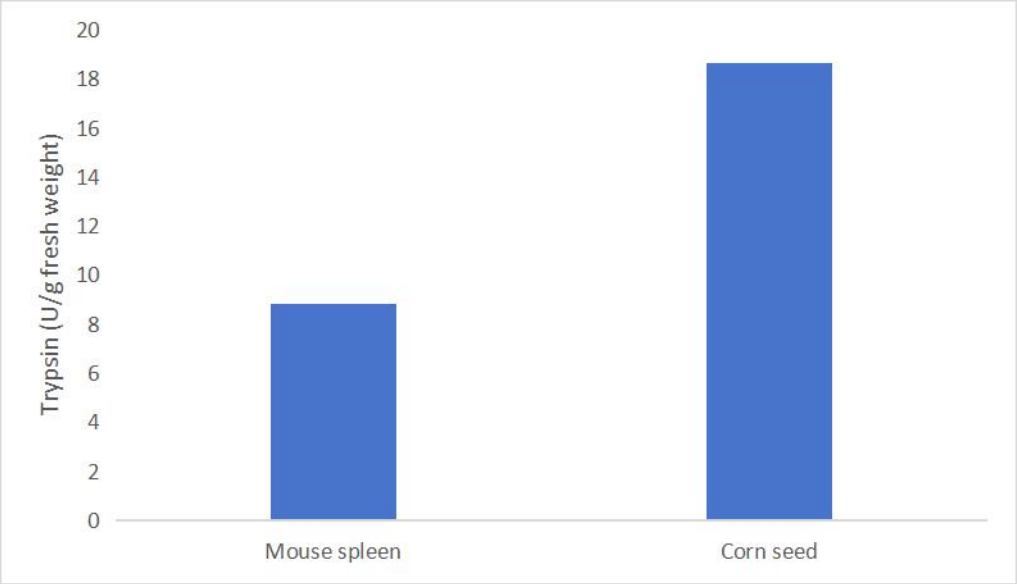

Figure 1. Determination of trypsase activity in mouse spleen and corn seed by this kit.

Recommended Products

Catalog No.	Product Name
KTB2270	CheKine™ Micro Acid Protease (ACP) Activity Assay Kit
KTB1380	CheKine™ Micro β-Amylase Activity Assay Kit
KTB1390	CheKine™ Micro Starch Branching Enzyme (SBE) Activity Assay Kit

Disclaimer

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