# CheKine™ Micro Chymotrypsin Activity Assay Kit

Cat #: KTB2330 Size: 48 T/48 S 96 T/96 S

| | | | |
|---|---|---|---|
| | **Micro Chymotrypsin Activity Assay Kit** | | |
| REF | **Cat #:** KTB2330 | LOT | **Lot #:** Refer to product label |
| | **Applicable samples:** Animal and Plant Tissues, Plasma, Serum or other Liquid samples | | |
| | **Storage:** Stored at 4℃ for 6 months, protected from light | | |

## Assay Principle

Chymotrypsin, also known as chymotrypsin, is a proteolytic enzyme secreted by the pancreas that can quickly break down denatured proteins. The function of chymotrypsin is similar to that of trypsin, but it has advantages such as strong decomposition ability, low toxicity, and minimal adverse reactions. In clinical practice, chymotrypsin is used for sputum dilution and is effective for both purulent and non purulent sputum; It is also used for wound healing after trauma surgery, such as cataract removal. Chymotrypsin catalyzes the hydrolysis of ATEE, and the product exhibits characteristic light absorption at 237 nm; Calculate chymotrypsin activity by measuring the rate of increase in 237 nm light absorption.

## Materials Supplied and Storage Conditions

| Kit components | Size | | 储存条件 |
|---|---|---|---|
| | 48 T | 96 T | |
| Reagent Ⅰ | 60 mL | 120 mL | 4℃ |
| Reagent Ⅱ | Powder×1 vial | Powder×1 vial | 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 ultraviolet spectrophotometer capable of measuring absorbance at 237 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips, 1.5 mL eppendorf tube
- Water bath, incubator, ice maker, centrifuge, magnetic stirrer
- Deionized water
- Homogenizer or mortar (for tissue samples)

## Reagent Preparation

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

**Reagent Ⅱ:** Prepared before use. 48 T add 12 mL deionized water, 96 T add 24 mL deionized water to dissolve thoroughly;

Unused reagents can be stored at 4℃ in the dark for 3 days.

## 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. 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 ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 237 nm. ultraviolet spectrophotometer was returned to zero with deionized water.
2. Incubate Reagent Ⅱ at 40℃ for 30 min.
3. Sample measurement. (The following operations are operated in the 96-well UV plate or microquartz cuvette)

| Reagent | Test Well (μL) | Blank Well (μL) |
|---|---|---|
| Supernatant | 20 | 0 |
| Reagent Ⅰ | 0 | 20 |
| Reagent Ⅱ | 200 | 200 |

Mix thoroughly and measure the absorbance value change within 4 min at 237 nm. The absorbance of test well and blank well were recorded as $A_{Test}$ and $A_{Blank}$ (timing starts from a stable increase in absorbance value). Calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$.

**Note: 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 $\Delta A_{Test}$ is less than 0.01, increase the sample quantity appropriately. If $\Delta A_{Test}$ is greater than 1.0, the sample can be appropriately diluted with Reagent Ⅰ, 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 chymotrypsin activity

(1) Calculated by sample protein concentration

Active unit definition: 1 increase in absorbance value catalyzed per min in 1 mg tissue protein at 25℃ is defined as a unit of enzyme activity.

Chymotrypsin (U/mg prot)=$\Delta A_{Test}\times V_{Total}\div(Cpr\times V_{sample})\div T$**=$2.75\times\Delta A_{Test}\div Cpr$**

(2) Calculated by fresh weight of samples

Active unit definition: 1 increase in absorbance value catalyzed per min in 1 g tissue at 25℃ is defined as a unit of enzyme activity.

Chymotrypsin (U/g fresh weigh)=$\Delta A_{Test}\times V_{Total}\div(W\times V_{sample}\div V_{Total\ Sample})\div T$**=$2.75\times\Delta A_{Test}\div W$**

(3) Calculated by volume of liquid samples

Active unit definition: 1 increase in absorbance value catalyzed per min in 1 mL serum at 25℃ is defined as a unit of enzyme activity.

Chymotrypsin (U/mL)=$\Delta A_{Test} \times V_{Total} \div V_{sample} \div T = 2.75 \times \Delta A_{Test}$

$V_{Total}$: total reaction volume, 0.22 mL; Cpr; sample protein concentration, mg/mL; $V_{Sample}$: sample volume added, 0.02 mL; $V_{Total\ Sample}$: Reagent I volume added, 1 mL; T: reaction time, 4 min; W: sample weight, g.

## Typical Data

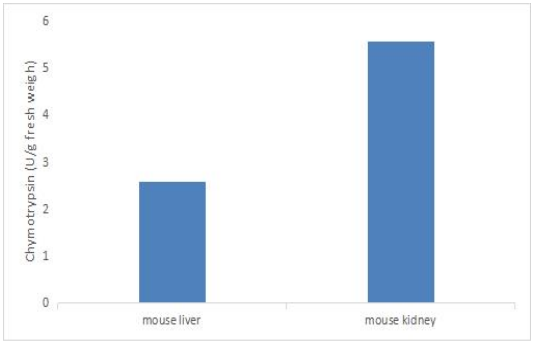


Figure 1. Determination of chymotrypsin activity in mouse liver and mouse kidney by this kit.

## 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. For your safety and health, please wear a lab coat and disposable gloves.