TECHNICAL MANUAL

Chymotrypsin Activity Colorimetric Assay Kit

- **SKU CODE:** MAES0525
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure the OD value

2. Intended use

This kit can measure chymotrypsin activity in tissue samples.

3. Detection principle

Chymotrypsin is a serine protease secreted by the pancreas, which is primarily used for protein decomposition during digestion. It specifically hydrolyzes peptide bonds of aromatic amino acids (such as phenylalanine, tryptophan, and tyrosine) in proteins to generate smaller peptides or amino acids for further absorption by the body. Additionally, chymotrypsin is widely used in protein analysis and research in the laboratory, especially for protein sequencing and structural analysis.

Detection principle: The substrate is converted to a colored product after chymotrypsin catalysis. The optical density (OD) value is detected at a wavelength of 405 nm, and the enzyme activity is calculated from the OD value of the standard.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	25 mL × 1 vial	50 mL × 1 vial	-20°C, 12 months, protected from light
Reagent 2	Substrate	0.5 mL × 1 vial	1 mL × 1 vial	-20°C, 12 months, protected from light
Reagent 3	5 mmol/L Standard Solution	1.5 mL × 1 vial	1.5 mL × 2 vials	-20°C, 12 months, protected from light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (400-410 nm, optimum wavelength: 405 nm), Incubator (37°C)

Reagents:

Normal saline (0.9% NaCl)

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For small volume reagents, please centrifuge before use to ensure sufficient reagent recovery.

6. Reagent preparation

1. Equilibrate all reagents to 25°C before use.
2. **Preparation of working solution:** Before testing, prepare sufficient working solution according to the number of test wells. For example, to prepare 200 µL of working solution, thoroughly mix 8 µL of substrate with 192 µL of buffer solution. Keep on ice during use, protected from light, and use within the same day.
3. **Preparation of 1 mmol/L standard solution:** Before testing, prepare sufficient 1 mmol/L standard solution. For example, to prepare 1000 µL of 1 mmol/L standard solution, thoroughly mix 200 µL of 5 mmol/L standard solution with 800 µL of buffer solution. The 1 mmol/L standard solution should be prepared fresh and stored protected from light.
4. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 1 mmol/L standard solution with buffer solution to create a serial dilution. The recommended dilution gradient is as follows: 0, 0.2, 0.3, 0.4, 0.6, 0.7, 0.8, 1 mmol/L.

7. Sample preparation

Tissue sample preparation:

1. Harvest the amount of tissue needed for each assay (initial recommendation: 20 mg).

2. Wash tissue in normal saline (0.9% NaCl).

3. Homogenize 20 mg tissue in 180 µL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

4. Centrifuge at 10,000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Sample dilution:

The recommended dilution factors for different samples are as follows (for reference only):

- 10% Mouse small intestine tissue homogenate: 40-50

- 10% Mouse pancreas tissue homogenate: 5-10

- 10% Mouse liver tissue homogenate: 5-10

Note: The diluent is normal saline (0.9% NaCl). For dilution of other sample types, please perform a pre-test to confirm the dilution factor.

8. Operating steps

1. Standard well: Add 20 µL of standard solution with different concentrations to the corresponding wells. Sample well: Add 20 µL of samples to sample wells.
2. Add 180 µL of working solution to each well.
3. Mix thoroughly with microplate reader for 5 s and measure the OD value of each well at 405 nm, recorded as A1. Incubate at 37°C for 30 min and measure the OD value of each well at 405 nm, recorded as A2. The standard curve is fitted using the A2 values of the standard wells.

9. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

2. Subtract the mean A_2 value of the blank (Standard #①) from all standard readings. This is the absolute A_2 value.

3. Plot the standard curve using the absolute A_2 value of standards and corresponding concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

Tissue sample calculation:

Definition: The amount of enzyme in 1 g tissue protein per 1 min that produces 1 µmol product at 37°C is defined as 1 unit.

Chymotrypsin activity (U/g_{prot}) = (ΔA_405 - b) ÷ a ÷ T × f ÷ C_{pr} × 1000

[Note]

ΔA_405: $\Delta A_405 = A_2 - A_1$

T: Reaction time, 30 min

f: Dilution factor of sample before test

C_{pr}: Concentration of protein in sample, g_{prot}/L

1000: 1 mmol/L = 1000 μmol/L

10. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three mouse liver samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.20	0.40	0.80
%CV	1.2	6.1	9.7

Recovery

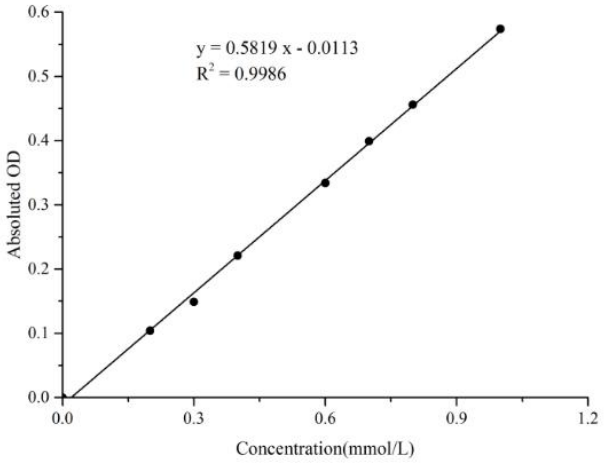
Three samples of high, middle, and low concentration were tested with 6 parallel replicates for each concentration to obtain an average recovery rate of 99%.

Sensitivity

The analytical sensitivity of the assay is 0.028 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only.



11. Appendix II Example Analysis

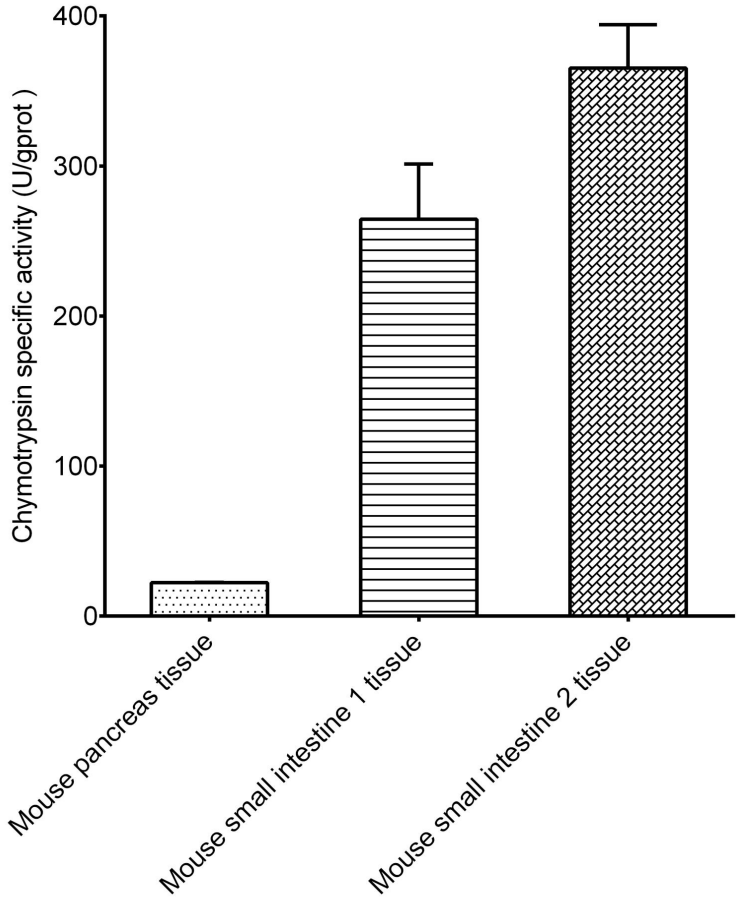
Example analysis:

Take 20 μL of 10% mouse small intestine tissue homogenate diluted 50 times and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.5819x – 0.0113, the A_1 of the sample well is 0.107, the A_2 of the sample well is 0.521, ΔA_405 = 0.521 - 0.107 = 0.414, the concentration of protein is 4.97 g_{prot}/L, and the calculation result is:

Chymotrypsin activity (U/g_{prot}) = (0.414 + 0.0113) ÷ 0.5819 ÷ 30 × 50 ÷ 4.97 × 1000 = 245.1 U/g_{prot}

Detection of 10% mouse pancreas tissue homogenate (protein concentration: 13.18 g_{prot}/L, diluted 5 times), 10% mouse small intestine tissue 1 homogenate (protein concentration: 4.97 g_{prot}/L, diluted 50 times) and 10% mouse small intestine tissue 2 homogenate (protein concentration: 7.27 g_{prot}/L, diluted 50 times) according to the protocol yielded the following results:



12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality

of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.