TECHNICAL MANUAL

Trypsin Activity Assay Kit

- **SKU CODE:** MAES0283
- **SIZE:** 96T
- **PRODUCT TYPE:** Colorimetric
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Key Features

Detection Range: 1.86-100.65 U/L

Measuring Instrument: Microplate reader (400-410 nm)

2. Intended use

This kit can be used to measure trypsin activity in animal tissue samples.

3. Detection principle

Trypsin catalyzes the hydrolysis of substrate to form p-nitroaniline, which exhibits characteristic light absorption at 400-410 nm wavelength. Since the absorbance of p-nitroaniline is proportional to its concentration, trypsin activity can be quantified by measuring the amount of p-nitroaniline produced per unit time.

4. Kit components & storage

Store all components under the conditions specified in the table above. Reagents from different kits cannot be mixed with each other. For small volume reagents, please centrifuge before use to ensure sufficient reagent recovery.

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	50 mL × 1 vial	50 mL × 2 vials	-20°C, 12 months
Reagent 2	Substrate	Powder × 1 vial	Power × 2 vials	-20°C, 12 months
Reagent 3	Standard	Powder × 1 vial	Power × 2 vials	-20°C, 12 months, shading light
Reagent 4	Diluent	2 mL × 1 vial	4 mL × 1 vial	-20°C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		

5. Materials prepared by users

Instruments

Microplate reader (400-410 nm, optimal wavelength: 405 nm), incubator

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.

2. Preparation of substrate working solution:

Dissolve one vial of substrate with 0.5 mL of diluent, mix well to dissolve completely. The prepared solution should be used within 4 hours.

3. Preparation of reaction working solution:

Before testing, prepare sufficient reaction working solution according to the number of test wells. For example, prepare 250 μL of reaction working solution by mixing 240 μL of buffer solution and 10 μL of substrate working solution. The reaction working solution should be prepared fresh.

4. Preparation of standard working solution:

Dissolve one vial of standard with 1 mL of diluent, mix well to dissolve completely. Store at -20°C for up to 7 days, protected from light.

5. Preparation of 1 mmol/L standard solution:

Before testing, prepare sufficient 1 mmol/L standard solution according to the number of test wells. For example, prepare 1000 μL of 1 mmol/L standard solution by mixing 50 μL of standard working solution and 950 μL of buffer solution. The 1 mmol/L standard solution should be prepared fresh and used within 4 hours.

6. 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 concentration series. The recommended dilution gradient is as follows: 0, 0.2, 0.3, 0.4, 0.6, 0.7, 0.8, 1 mmol/L. Reference preparation is as follows:

Item	1	2	3	4	5	6	7	8
Concentration (mmol/L)	0	0.2	0.3	0.4	0.6	0.7	0.8	1
10 mmol/L standard (μL)	0	40	60	80	120	140	160	200
Buffer Solution (μL)	200	160	140	120	80	60	40	0

7. Sample preparation

Tissue sample

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).
2. Wash tissue in cold PBS (0.01 M, pH 7.4).
3. Homogenize 20 mg tissue in 180 μL buffer solution with a dounce homogenizer at 4°C.
4. Centrifuge at 10,000×g for 10 minutes to remove insoluble material. Collect supernatant and keep on ice for detection.
5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample

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

Please perform a pretest to confirm the appropriate dilution factor.

Sample type	Dilution factor
10% Mouse small intestine tissue homogenate	2-5
10% Mouse large intestine tissue homogenate	2-5
10% Rat intestine tissue homogenate	2-5
10% Rat chyme tissue homogenate	2-5

8. Operating steps

1. Standard wells: Add 10 μL of standards with different concentrations to the corresponding wells. Sample wells: Add 10 μL of sample to the corresponding wells.
2. Add 160 μL of reaction working solution to standard wells and sample wells. Mix fully for 5 seconds with microplate reader. Measure the OD value of each well at 405 nm, recorded as A1.
3. Incubate at 37°C for 10 minutes, then measure the OD value of each well at 405 nm, recorded as A2.

9. Calculation

The standard curve

1. Average the duplicate readings for each standard.
2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absolute OD value.
3. Plot the standard curve using the absolute OD value of standards and corresponding concentration as y-axis and x-axis respectively. Create the

$$y = ax + b$$

standard curve (y = ax + b) with graphing software (or Excel).

Definition

The amount of trypsin in 1 g tissue protein that catalyzes the substrate to produce 1 μmol p-nitroaniline per minute at 37°C is defined as 1 unit.

Trypsin activity (U/gprot) = (ΔA_{405} - b) ÷ a ÷ T ÷ Cpr × f × 1000

[Note]

ΔA_{405}: Absolute OD (A_2 - A_1).

T: The time of reaction, 10 min.

f: Dilution factor of sample before test.

Cpr: Concentration of protein in sample, gprot/mL.

1000: 1 mmol/L = 1000 μmol/L.

10. Appendix I Performance Characteristics

Intra-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	5.60	26.80	76.40
%CV	5.1	3.9	4.5

Inter-assay Precision

Three rat intestine tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	5.60	26.80	76.40
%CV	11.2	7.2	9.8

Recovery

Three samples of high, medium, and low concentrations were tested with 6 replicates each to determine recovery rate, yielding an average recovery rate of 106%.

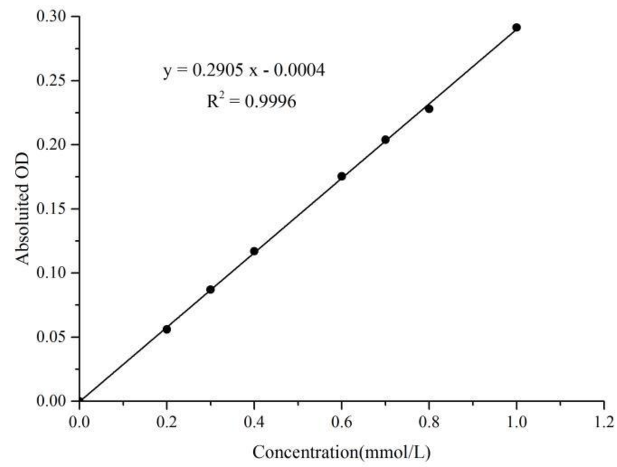
Standard 1	Standard 2	Standard 3
0.24	0.5	0.73
0.3	0.5	0.8
113	100	105

Sensitivity

The analytical sensitivity of the assay is 1.86 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.

2. Standard curve

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



Concentration (mmol/L)	**0**	**0.2**	**0.3**	**0.4**	**0.6**	**0.7**	**0.8**	**1.0**
Average OD	0.367	0.423	0.454	0.484	0.543	0.571	0.595	0.659
Absolute OD	0	0.056	0.087	0.117	0.176	0.204	0.228	0.292

11. Appendix II Example Analysis

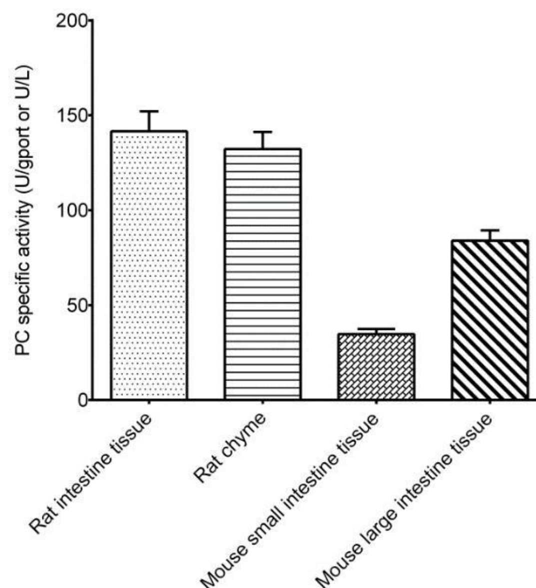
Example analysis

For 10% mouse small intestine tissue homogenate, diluted 2.5 times, take 10 μL and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.2905x - 0.0004, A_1 of sample is 0.555, A_2 of sample is 0.606, ΔA_{405} = A_2 - A_1 = 0.606 - 0.555 = 0.051, protein concentration in sample is 1.32 gprot/L, and the calculation result is:

Trypsin activity (U/gprot) = (0.051 + 0.0004) ÷ 0.2905 ÷ 10 × 2.5 ÷ 1.324 × 1000 = 33.42 U/gprot

Detection of 10% mouse small intestine tissue homogenate (protein concentration: 1.32 gprot/L), 10% mouse large intestine tissue homogenate (protein concentration: 1.22 gprot/L), 10% rat intestine tissue homogenate (protein concentration: 0.98 gprot/L), and 10% rat chyme tissue homogenate (protein concentration: 1.76 gprot/L) according to the protocol yielded the following results:



12. Statement

1. Assay Genie provides this kit for research use only and is not responsible for any problems or legal responsibilities arising from using the kit for clinical diagnosis or other purposes.

2. Please read the instructions carefully and calibrate instruments before experiments. Follow the instructions strictly during experiments.

3. Protective measures must be taken by wearing lab coat and latex gloves.

4. If the substance concentration is not within the detection range, additional dilution or concentration should be performed on the sample.

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

6. The experimental results are closely related to reagent conditions, operations, environment, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption caused by using the assay kits. It is advisable to calculate possible sample usage and reserve sufficient samples before use.

Notes:

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.