TECHNICAL MANUAL

Pepsin Activity Assay Kit

- **SKU CODE:** MAES0284
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Intended use

This kit can measure pepsin activity in gastric and intestinal tissue samples.

2. Detection principle

Pepsin can catalyze substrate hydrolysis, and the hydrolysate reacts with the chromogenic agent under alkaline conditions to form a blue substance. The blue substance has a maximum absorption peak at 660 nm, and the color intensity is proportional to the activity of pepsin. The enzyme activity can be calculated by OD value.

3. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	55 mL × 2 vials	-20 °C, 12 months
Reagent 2	Precipitating Agent	50 mL × 1 vial	-20 °C, 12 months
Reagent 3	Chromogenic Agent A	20 mL × 1 vial	-20 °C, 12 months
Reagent 4	Chromogenic Agent B	10 mL × 1 vial	-20 °C, 12 months, shading light
Reagent 5	Substrate	Powder × 2 vials	-20 °C, 12 months, shading light
Reagent 6	Substrate Solvent	12 mL × 1 vial	-20 °C, 12 months
Reagent 7	1 mg/mL Standard Solution	1.5 mL × 1 vial	-20 °C, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

4. Materials prepared by users

Instruments:

Microplate reader (650-670 nm, optimum wavelength: 660 nm), Incubator

Reagents:

Normal saline (0.9% NaCl)

5. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of substrate working solution::** Dissolve one vial of substrate with 5 mL of substrate solvent. Dissolve completely in 75 °C water bath for 30 min. Store at 2-8 °C for 2 days.
3. **Preparation of 60 μg/mL standard solution::** Before testing, prepare sufficient 60 μg/mL standard solution according to the test wells. For example, prepare 1000 μL of 60 μg/mL standard solution (mix well 940 μL of buffer solution and 60 μL of 1 mg/mL standard solution). The prepared solution should be prepared on spot.
4. **Preparation of measuring working solution::** Before testing, prepare sufficient measuring working solution according to the test wells. For example, prepare 320 μL of measuring working solution (mix well 195 μL of buffer solution and 125 μL of substrate working solution). The prepared solution should be prepared on spot.
5. **The preparation of standard curve::** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 60 μg/mL standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 12, 18, 24, 36, 42, 48, 60 μg/mL.

6. Sample preparation

Sample preparation

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 30 mg).

2. Wash tissue in cold PBS (0.01 M, pH 7.4).

3. Homogenize 30 mg tissue in 120 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4 °C.

4. Centrifuge at 10000 xg for 10 min at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection.

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

Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

20% Rat gastric tissue homogenate: 1

20% Mouse gastric tissue homogenate: 1

20% Rat intestinal tissue homogenate: 1

20% Mouse intestinal tissue homogenate: 1

Note: The diluent is buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

7. Operating steps

Enzymatic reaction

1. Control tube: Take 40 µL of sample to the 2 mL EP tube.

Sample tube: Take 40 µL of sample to the 2 mL EP tube.

2. Add 400 µL of precipitating agent to control tube.

3. Add 200 µL of measuring working solution to control tube and sample tube. Mix fully and incubate at 37 °C for 10 min.

4. Add 400 µL of precipitating agent to sample tube.

5. Mix fully and incubate at 37 °C for 10 min, then centrifuge at 3500 rpm for 10 min and take the supernatant for detection.

Chromogenic reaction

1. Standard well: Take 60 µL of standards with different concentrations to the corresponding wells.

Control well: Take 60 µL of supernatant of control tube to the corresponding wells.

Sample well: Take 60 µL the supernatant of sample tube to the corresponding wells.

2. Add 150 µL of chromogenic agent A to each well.

3. Add 60 µL of chromogenic agent B to each well.

4. Mix fully with microplate reader and incubate at 37 °C for 10 min.

5. Measure the OD value of each well at 660 nm with microplate reader.

8. Calculation

The standard curve:

1. Average the duplicate reading 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 by using absolute OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

Tissue sample:

Definition: The amount of pepsin in 1 mg tissue protein per 1 min that catalyzes substrate to produce 1 μg product at 37 ℃ is defined as 1 unit.

Pepsin activity (U/mgprot) = (ΔA_660 - b) ÷ a ÷ T × (V_1 ÷ V_2) ÷ C_{pr} × f

[Note]

ΔA_660: (OD_{Sample} - $OD_{Control}$).

T: The time of enzymatic reaction, 10 min.

V_1: The volume of enzymatic reaction, 0.64 mL.

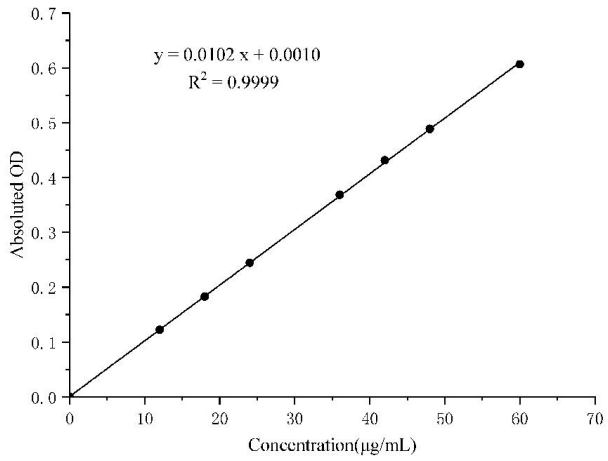
V_2: The volume of sample in enzymatic reaction, 0.04 mL.

C_{pr}: The concentration of protein in sample, mgprot/mL.

f: Dilution factor of sample before test.

9. Appendix I Performance Characteristics

Parameter:



2. 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), so the standard curve and data are provided as below for reference only:

Intra-assay Precision

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

Inter-assay Precision

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

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 95%.

Sensitivity

The analytical sensitivity of the assay is 0.63 U/mL. 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.

10. Appendix II Example Analysis

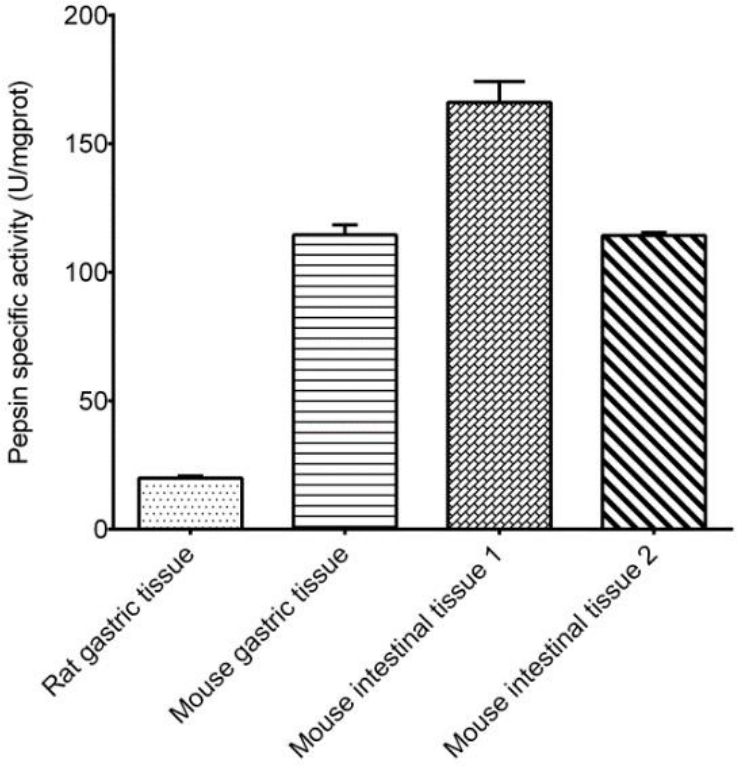
Example analysis:

For 20% rat gastric tissue homogenate, take 40 μL of sample and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0102x + 0.001, the average OD value of the control is 0.379, the average OD value of the sample is 0.414, the concentration of protein in sample is 0.268 mgprot/mL, and the calculation result is:

Pepsin activity (U/mgprot) = (0.414 - 0.379 - 0.001) ÷ 0.0102 ÷ 10 × 1 × (0.64 ÷ 0.04) ÷ 0.268 = 19.9 U/mgprot

Detect 20% rat gastric tissue homogenate (the concentration of protein is 0.268 mgprot/mL), 20% mouse gastric tissue homogenate (the concentration of protein is 0.251 mgprot/mL), 20% mouse intestinal tissue 1 homogenate (the concentration of protein is 0.216 mgprot/mL), 20% mouse intestinal tissue 2 homogenate (the concentration of protein is 0.281 mgprot/mL) according to the protocol, the result is as follows:



11. 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.