TECHNICAL MANUAL

Sucrase Activity Assay Kit

- **SKU CODE:** MAES0192
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Operating steps
- Calculation

2. Intended use

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

3. Detection principle

Sucrase catalyzes its substrate (sucrose) to produce glucose, which generates hydrogen peroxide under the action of glucose oxidase. Hydrogen peroxide reacts with chromogenic agent to produce a red substance, which has a strong absorption peak at 505 nm. Within a certain concentration range, the absorbance is proportional to glucose concentration. Therefore, the activity of sucrase can be calculated by measuring the OD value at 505 nm.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Substrate	Powder x 1 vial	2-8 °C, 12 months
Reagent 2	Buffer Solution	10 mL x 1 vial	2-8 °C, 12 months
Reagent 3	Phenol Solution	12 mL x 1 vial	2-8 °C, 12 months, shading light
Reagent 4	Enzyme Solution	12 mL x 1 vial	2-8 °C, 12 months, shading light
Reagent 5	Stop Solution	Powder x 1 vial	2-8 °C, 12 months
Reagent 6	50 mmol/L Glucose Standard	1 mL x 1 vial	2-8 °C, 12 months
	Microplate	96 wells	No requirement

Item	Component	Size (96 T)	Storage
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Micropipette, Vortex mixer, Centrifuge, Microplate reader (500-520 nm, optimum wavelength: 505 nm)

Reagents:

Double distilled water, PBS (0.01 M, pH 7.4)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of substrate working solution:** Dissolve one vial of substrate with 8 mL of buffer solution, mix well to dissolve. Store at 2-8 °C for 7 days.
3. **Preparation of stop working solution:** Dissolve one vial of stop solution with 5 mL of ultrapure water, mix well to dissolve. Store at 2-8 °C for 7 days.
4. **Preparation of chromogenic solution:** For each well, prepare 200 µL of chromogenic solution (mix well 100 µL of phenol solution and 100 µL of enzyme solution). The chromogenic solution should be prepared fresh.
5. **The preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 50 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 1, 2, 5, 10, 15, 20, 25 mmol/L.

7. Sample preparation

1. **Tissue sample preparation:** 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 80 µL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4 °C. 4. Centrifuge at 10,000 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.

2. **Dilution of sample:** The recommended dilution factor for different samples is as follows (for reference only): 20% Rat ileum tissue homogenate: 1; 20% Rat stomach tissue homogenate: 1; 20% Rat liver tissue homogenate: 1. Note: The diluent is PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

8. The key points of the assay

1. Control the time of enzymatic reaction strictly.

2. Avoid contaminating enzyme solution when preparing chromogenic solution.

9. Operating steps

1. Standard tube: Take 25 μL of standards with different concentrations into 1.5 mL EP tubes. Sample tube: Take 25 μL of sample into 1.5 mL EP tubes. Control tube: Take 50 μL of substrate working solution into 1.5 mL EP tubes.

2. Add 50 μL of substrate working solution into the standard tubes and sample tubes.

3. Mix fully and incubate at 37 °C for 20 min.

4. Add 25 μL of stop working solution into each tube and mix fully. Add 25 μL of sample into control tubes.

5. Mix fully, centrifuge at 1,780 xg for 10 min and take 8 μL of supernatant from each tube to the corresponding wells.

6. Add 200 μL of chromogenic solution into each well.

7. Mix fully with microplate reader for 10 s, incubate at 37 °C for 15 min. Measure the OD values of each well at 505 nm with microplate reader.

10. 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 1 nmol sucrose hydrolyzed by 1 g tissue protein per minute at 37 °C is defined as 1 activity unit.

Sucrase activity (U/mgprot) = (ΔA - b) ÷ a ÷ T × 1000* × f ÷ C_{pr}

[Note]

ΔA: OD_{Sample} – $OD_{Control}$.

T: Enzymatic reaction time, 20 min.

1000*: 1 μmol = 1000 nmol.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in tissue sample, mgprot/mL.

Intra-assay Precision

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

Inter-assay Precision

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

Recovery

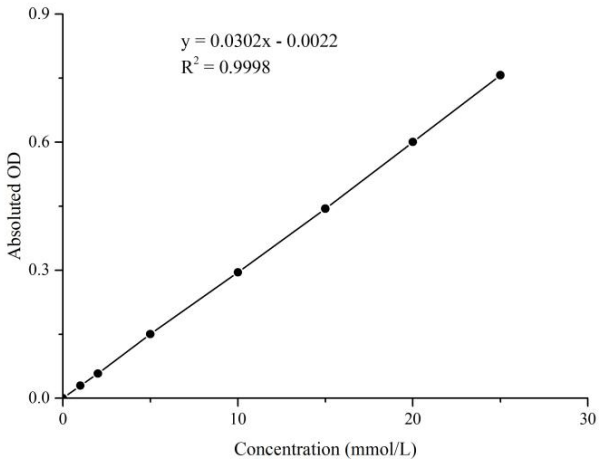
Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 100%.

Sensitivity

The analytical sensitivity of the assay is 20 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.

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:



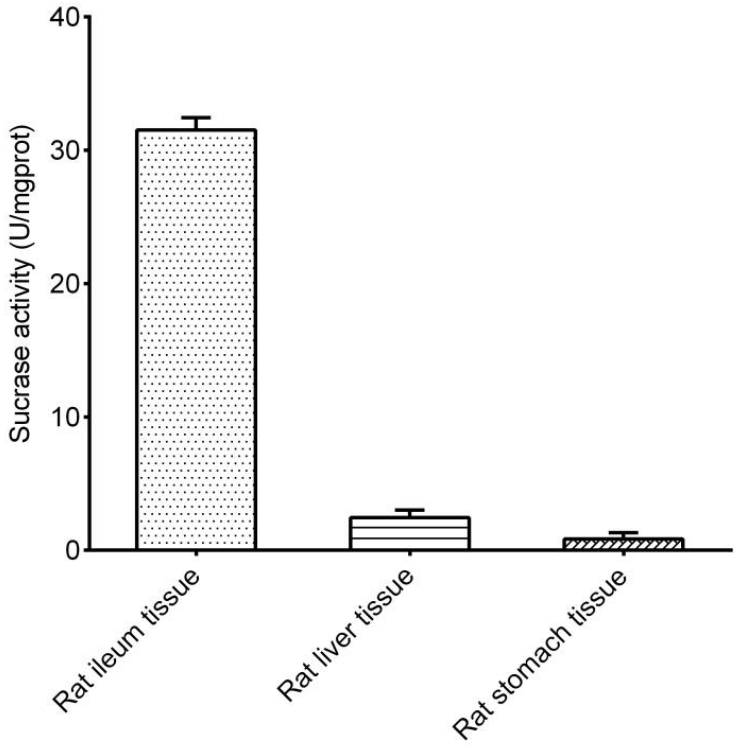
11. Appendix II Example Analysis

Example analysis:

For rat ileum tissue, take 25 μL of rat ileum tissue supernatant and carry the assay according to the operation table. The results are as follows: standard curve: y = 0.0306x + 0.0025, the average OD value of the sample is 0.204, the average OD value of the control is 0.079, the concentration of protein in sample is 6.48 mgprot/mL, and the calculation result is:

Sucrase activity (U/mgprot) = (0.204-0.079-0.0025) ÷ 0.0306 ÷ 20 × 1000 ÷ 6.48 = 30.89 U/mgprot

Detect 20% rat ileum tissue homogenate (the concentration of protein is 6.48 mgprot/mL), 20% rat liver tissue homogenate (the concentration of protein is 23.65 mgprot/mL) and 20% rat stomach tissue homogenate (the concentration of protein is 5.89 mgprot/mL) according to the protocol, the result is as follows:



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.