TECHNICAL MANUAL

Fructose Fluorometric Assay Kit

- **SKU CODE:** MAES0289
- **SIZE:** 96 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
- Incubate with enzyme working solution
- Add chromogenic/substrate working solution
- Measure fluorescence intensity

2. Intended use

This kit can be used to measure fructose content in serum, plasma, and tissue samples.

3. Detection principle

Fructose is the most common hexoketose, which is often present in large amounts as a free monosaccharide in fruits and honey. Extensive epidemiological data and experimental studies have shown that excessive fructose intake may be an important factor in the increased incidence of metabolic diseases.

Fructose can produce a specific product under the action of enzymes, which reacts with the chromogenic agent to produce a fluorescent substance. The fluorescence intensity is measured at an excitation wavelength of 530 nm and emission wavelength of 590 nm.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	50 mL x 1 vial	-20 °C, 12 months
Reagent 2	Matrix Solution	50 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 3	Enzyme Reagent	Powder x 8 vials	-20 °C, 12 months, shading light
Reagent 4	Chromogenic Agent	Powder x 8 vials	-20 °C, 12 months, shading light

Item	Component	Size (96 T)	Storage
Reagent 5	Substrate	Powder x 8 vials	-20 °C, 12 months, shading light
Reagent 6	Accelerant	1 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 7	5 mmol/L Standard Solution	1.6 mL x 1 vial	-20 °C, 12 months
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Fluorescence microplate reader (Ex/Em=530 nm/590 nm), Incubator (37 °C), Vortex mixer

Reagents:

Normal saline (0.9% NaCl)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of enzyme working solution:** Dissolve one vial of enzyme reagent with 1.125 mL of buffer solution, mix well to dissolve, and add 2 µL of accelerant to mix well. Store at 2-8 °C for 1 week protected from light.
3. **Preparation of chromogenic working solution:** Dissolve one vial of chromogenic agent with 1.5 mL of matrix solution, mix well to dissolve. Store at 2-8 °C for 3 days protected from light.
4. **Preparation of substrate working solution:** Dissolve one vial of substrate with 1.5 mL of matrix solution, mix well to dissolve. Store at 2-8 °C for 3 days protected from light.
5. **Preparation of 500 µmol/L standard solution:** Before testing, prepare sufficient 500 µmol/L standard solution according to the test wells. For example, prepare 1000 µL of 500 µmol/L standard solution by mixing 100 µL of 5 mmol/L standard

solution with 900 µL of double distilled water. Store at 2-8 °C for a week protected from light.

6. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 500 µmol/L standard solution with double distilled water to serial concentrations. The recommended dilution gradient is: 0, 100, 200, 250, 300, 350, 400, 500 µmol/L.

7. Standard dilution table

Item	1	2	3	4	5	6	7	8
Concentration (µmol/L)	0	100	200	250	300	350	400	500
500 µmol/L standard (µL)	0	40	80	100	120	140	160	200
Double distilled water (µL)	200	160	120	100	80	60	40	0

8. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, serum or plasma can be stored at -80 °C for a month.

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 normal saline (0.9% NaCl) 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 (MAES0177; MAES0126).

Sample dilution:

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

10% Watermelon tissue homogenate: 40-90

10% Cantaloupe tissue homogenate: 30-90

10% Orange tissue homogenate: 20-80

10% Mango tissue homogenate: 30-90

10% Grape tissue homogenate: 60-200

10% Pineapple tissue homogenate: 15-40

10% Mouse liver tissue homogenate: 1

Mouse serum: 1

Human serum: 1

Rabbit serum: 1

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

9. Operating steps

1. Standard well: Add 20 µL of standard with different concentrations into the well. Sample well: Add 20 µL of sample into the well. Control well: Add 20 µL of sample into the well.
2. Add 60 µL of enzyme working solution into each well.
3. Mix thoroughly with microplate reader for 3 s. Incubate at 37 °C for 90 min protected from light.
4. Add 100 µL of chromogenic working solution into the standard well and sample well.
5. Add 100 µL of substrate working solution into the control well.
6. Mix thoroughly with microplate reader for 5 s. Incubate at 37 °C for 30 min. Measure the fluorescence intensity at the excitation wavelength of 530 nm and emission wavelength of 590 nm.

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

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

The sample:

1. Serum (plasma) sample:

Fructose content (μmol/L) = (ΔF - b) / a × f

2. Tissue sample:

Fructose content (mmol/gprot) = (ΔF - b) / a ÷ C_{pr} ÷ 1000 × f

[Note]

ΔF: The absolute fluorescence value of sample, F_{sample} – $F_{control}$.

1000: 1 mmol = 1000 μmol.

f: Dilution factor of sample before tested.

C_{pr}: Concentration of protein in tissue sample, gprot/L.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision Data

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	50.00	150.00	350.00
%CV	1.7	2.3	3.1

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision Data

Parameters	Sample 1	Sample 2	Sample 3
Mean (µmol/L)	50.00	150.00	350.00
%CV	3.2	5.2	7.3

Recovery

Three samples of high, medium, and low concentrations were tested 6 times in parallel to obtain an average recovery rate of 101%.

Recovery Data

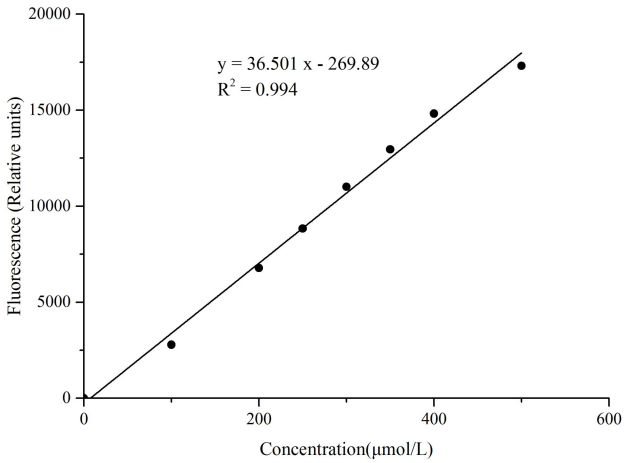
Parameters	Standard 1	Standard 2	Standard 3
Expected Conc.(µmol/L)	50	150	350
Observed Conc.(µmol/L)	48.5	142.5	388.5
Recovery rate (%)	97	95	111

Sensitivity

The analytical sensitivity of the assay is 0.72 µmol/L. This was determined by adding two standard deviations to the mean fluorescence value obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the fluorescence 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:



Standard curve data

Concentration (μmol/L)	Fluorescence value 1	Fluorescence value 2	Average fluorescence value	Absolute fluorescence value
0	490	497	493	0
100	3163	3401	3282	2789
200	7128	7417	7273	6779
250	8990	9664	9327	8834
300	11105	11908	11506	11013
350	13218	13678	13448	12955
400	14882	15740	15311	14818
500	17205	18396	17800	17307

12. Appendix II Example Analysis

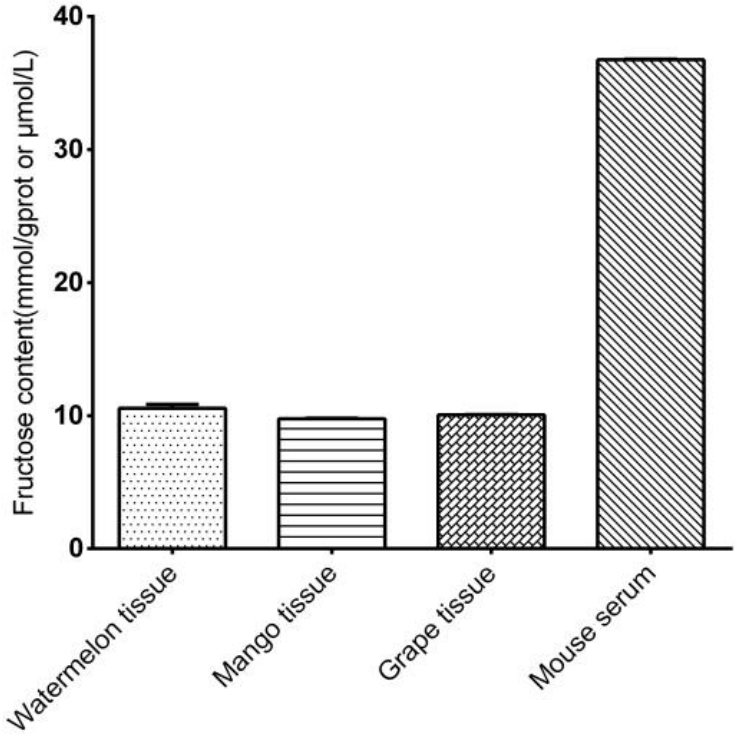
Example analysis:

Dilute 10% grape tissue homogenate 30 times, take 20 μL of diluted sample and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 36.501x - 269.89, the fluorescence value of the sample is 17,288, the fluorescence value of the control is 686, the concentration of protein in sample is 1.42 gprot/L, and the calculation result is:

Fructose content (mmol/gprot) = (17,288 – 686 + 269.89) ÷ 36.501 ÷ 1.42 ÷ 1000 × 30 = 9.77 mmol/gprot

Detect 10% watermelon tissue homogenate (protein concentration 1.97 gprot/L, diluted 40 times), 10% mango tissue homogenate (protein concentration 1.42 gprot/L, diluted 30 times), 10% grape tissue homogenate (protein concentration 3.27 gprot/L, diluted 60 times) and mouse serum according to the protocol, the result is as follows:



13. 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.