TECHNICAL MANUAL

Adipogenesis Fluorescence Assay Kit

- **SKU CODE:** MAES0252
- **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
- Measure fluorescence intensity

2. Intended use

This kit can be used to measure the choline/acetylcholine content in tissue and cell samples.

3. Detection principle

Triglycerides are converted by enzyme to produce hydrolytic products, which are catalyzed by the enzyme to produce fluorescent substances. The triglyceride accumulation can be calculated by measuring the fluorescence intensity at an excitation wavelength of 535 nm and an emission wavelength of 587 nm.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Enzyme Working Solution	25 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 2	Extraction Solution	50 mL × 1 vial	2-8°C, 12 months
Reagent 3	Probe	0.5 mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	1 mmol/L Standard	0.5 mL × 1 vial	2-8°C, 12 months, shading light
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Micropipettor, Incubator, Centrifuge, Fluorescence microplate reader (Ex/Em=535 nm/587 nm).

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of chromogenic working solution:** For each well, prepare 200 µL of chromogenic working solution by mixing 196 µL of enzyme working solution and 4 µL of probe. The chromogenic working solution should be prepared fresh and protected from light.
3. **Preparation of 40 µmol/L standard solution:** Dilute 20 µL of 1 mmol/L standard solution with 480 µL of extraction solution and mix thoroughly. Store at 2-8°C for up to 3 days, protected from light.
4. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 40 µmol/L standard with extraction solution to create serial concentrations. The recommended dilution gradient is as follows: 0, 8, 16, 20, 24, 32 µmol/L.

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 extraction solution with a dounce homogenizer at 4°C.

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

5. Meanwhile, determine the protein concentration of supernatant.

6. Place on ice for 30 min.

7. Heat the supernatant at 90-100°C for 30 min to ensure the lipids are completely dissolved in extraction solution. Cool to room temperature for detection. If turbidity occurs, centrifuge again and use the supernatant for determination. (Due to the high lipid content in tissue samples, it is recommended to perform a pre-experiment with 2-3 samples to determine the appropriate dilution ratio using extraction solution as diluent.)

Cell (adherent or suspension) samples:

1. Harvest the number of cells needed for each assay (initial recommendation: 1×10^4 cells).

2. Wash cells with PBS (0.01 M, pH 7.4).

3. Homogenize 1×10^4 cells in 200 µL extraction solution using an ultrasonic cell disruptor at 4°C.

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

5. Meanwhile, determine the protein concentration of supernatant.

6. Heat the supernatant at 90-100°C for 30 min to ensure the lipids are completely dissolved in extraction solution. Cool to room temperature for detection.

Recommended dilution factors for different samples (for reference only):

- 10% Porcine heart tissue homogenate: 3-6

- 10% Porcine liver tissue homogenate: 8-10

- CHO cell (10^6): 1

- 293T cell (10^6): 1

Note: Use extraction solution as diluent. For other sample types, perform a pretest to confirm the dilution factor.

8. The key points of the assay

It is necessary to wash the preparation equipment several times before preparing the chromogenic working solution to prevent impurity contamination.

9. Operating steps

1. Standard wells: Add 20 µL of standard with different concentrations to the corresponding wells. Sample wells: Add 20 µL of sample supernatant to the corresponding wells.
2. Add 200 µL chromogenic working solution to each well.
3. Mix well and incubate at 37°C for 5 min.
4. Measure the fluorescence intensity of each well at an excitation wavelength of 535 nm and an emission wavelength of 587 nm.

10. Calculation

The standard curve:

1. Average the duplicate readings 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 using absolute fluorescence values of standards and corresponding concentrations as y-axis and x-axis respectively. Create the standard curve (y = ax + b) using graph software or Excel.

The sample:

Tissue and cell samples:

Triglyceride content (μmol/g_{prot}) = (ΔF - b) ÷ a ÷ C_{pr} × f

Where:

ΔF: F_{Sample} - F_{Blank} (F_{Blank} is the fluorescence value when the standard concentration is 0)

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

f: Dilution factor of sample before test

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three porcine heart tissue 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 (μmol/L)	2.00	15.00	25.00
%CV	9.7	7.9	7.6

Recovery

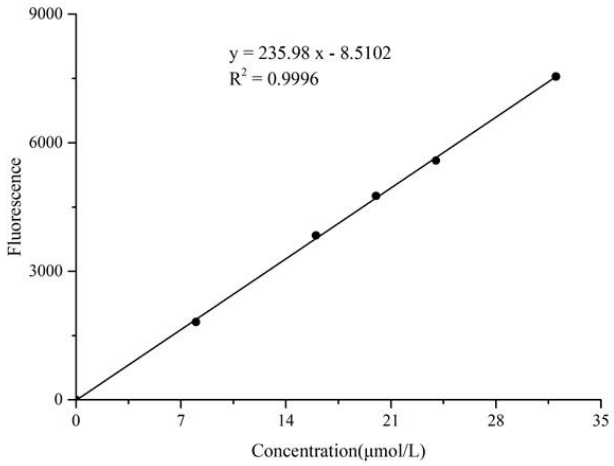
Three samples of high, middle, and low concentrations were tested with 6 parallel measurements for each concentration to obtain an average recovery rate of 98%.

Sensitivity

The analytical sensitivity of the assay is 1.4 μmol/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 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 provided below are for reference only:



12. Appendix II Example Analysis

Example analysis:

For porcine heart tissue, take 10% tissue homogenate supernatant, dilute 5-fold, then perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 235.98x - 8.5102

Average fluorescence value of sample well: 9614

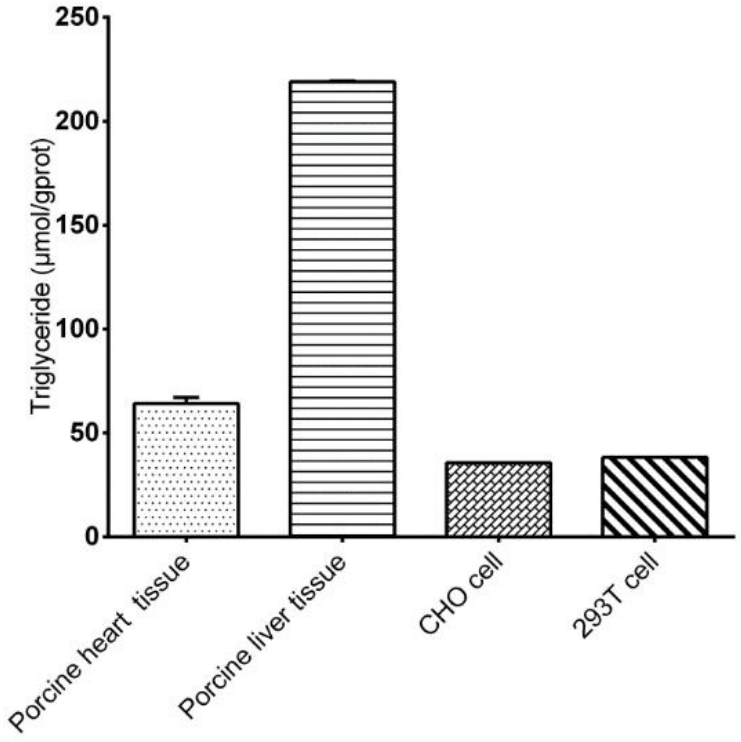
Average fluorescence value of blank well: 2121

Protein concentration: 2.56 g_{prot}/L

Calculation result:

Triglyceride content (μmol/g_{prot}) = (9614 - 2121 + 8.5102) ÷ 235.98 ÷ 2.56 × 5 = 62.08 μmol/g_{prot}

Detection of 10% porcine heart tissue homogenate (protein concentration: 2.56 g_{prot}/L, diluted 5-fold), 10% porcine liver tissue homogenate (protein concentration: 5.67 g_{prot}/L, diluted 5-fold), CHO cells (protein concentration: 0.98 g_{prot}/L), and 293T cells (protein concentration: 1.21 g_{prot}/L) according to the protocol:



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.

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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.