TECHNICAL MANUAL

Fatty Acid Amide Hydrolase (FAAH) Activity Fluorometric Assay Kit

- **SKU CODE:** MAES0323
- **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 substrate
- Measure fluorescence intensity

2. Intended use

This kit can be used to measure fatty acid amide hydrolase (FAAH) activity in animal tissue and cell samples.

3. Detection principle

Fatty acid amide hydrolase (FAAH) is a membrane-binding protein belonging to the serine hydrolase family. FAAH hydrolyzes many important endogenous fatty acid amides, including endocannabinoid (AEA) and palmitoyl glycolamide (PEA). FAAH plays a key role in controlling cannabinoid signaling by hydrolyzing 2-arachidonyl glycerol in AEA and certain tissues.

Detection principle: FAAH hydrolyzes a non-fluorescent substrate, releasing a fluorophore. Fluorescence is detected at an excitation wavelength of 340 nm and an emission wavelength of 440 nm. Because FAAH is a serine protease, FAAH-specific inhibitors are used in the kit to inhibit enzyme activity and exclude the effects of non-specific enzyme activity.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	55 mL x 1 vial	-20 °C, 12 months, protect from light
Reagent 2	Substrate	0.16 mL x 1 vial	-20 °C, 12 months, protect from light

Item	Component	Size (96 T)	Storage
Reagent 3	Inhibitor	0.08 mL x 1 vial	-20 °C, 12 months, protect from light
Reagent 4	1 mmol/L Standard Solution	0.4 mL x 1 vial	-20 °C, 12 months, protect from light
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments: Fluorescence microplate reader (Ex/Em=340 nm/440 nm), Incubator (37 °C).

6. Reagent preparation

1. Equilibrate all reagents to 25 °C before use.
2. **Preparation of substrate working solution:** Before testing, prepare sufficient substrate working solution according to the number of test wells. For example, to prepare 500 µL of substrate working solution, thoroughly mix 5 µL of substrate with 495 µL of buffer solution. The substrate working solution should be prepared fresh and used within 1 day.
3. **Preparation of inhibitor working solution:** Before testing, prepare sufficient inhibitor working solution according to the number of test wells. For example, to prepare 1000 µL of inhibitor working solution, thoroughly mix 5 µL of inhibitor with 995 µL of buffer solution. Store at -20 °C and use within 2 days.
4. **Preparation of 2 µmol/L standard solution:** Before testing, prepare sufficient 2 µmol/L standard solution according to the number of test wells. For example, to prepare 2500 µL of 2 µmol/L standard solution, thoroughly mix 5 µL of 1 mmol/L standard solution with 2495 µL of buffer solution. The 2 µmol/L standard solution should be prepared fresh and used within 1 day.
5. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 2 µmol/L standard solution with buffer solution to create a serial dilution. The recommended dilution gradient is as follows: 0, 0.25, 0.5, 0.75, 1, 1.5, 1.75, 2 µmol/L.

7. Sample preparation

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 180 µL buffer solution with a Dounce homogenizer at 4 °C.

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

Cell (adherent or suspension) sample preparation:

1. Harvest the number of cells needed for each assay (initial recommendation: 1 x 10^6 cells).

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

3. Homogenize 1 x 10^6 cells in 200 µL buffer solution with an ultrasonic cell disruptor at 4 °C.

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

Sample dilution:

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

- 10% Mouse liver tissue homogenate: 1:1

- 10% Mouse kidney tissue homogenate: 1:1

- 10% Mouse brain tissue homogenate: 1:1

- 10% Mouse lung tissue homogenate: 1:1

- 1 x 10^6 293T cells: 1:1

Note: The diluent is buffer solution. For dilution of other sample types, please perform a pre-test to confirm the dilution factor.

8. Operating steps

1. Standard wells: Add 180 µL of standard with different concentrations into corresponding wells respectively. Total enzyme wells: Add 20 µL of sample into the wells. Non-specific enzyme wells: Add 20 µL of sample into the wells.
2. Enzyme wells: Add 30 µL of buffer solution to the wells. Non-enzyme wells: Add 30 µL of inhibitor working solution to the wells.
3. Incubate at 25 °C for 5 min protected from light.
4. Add 130 µL substrate working solution to the enzyme wells and non-enzyme wells.

5. Mix thoroughly with microplate reader for 5 seconds and incubate at 37 °C for 25 min protected from light. Measure the fluorescence intensity at excitation wavelength of 340 nm and emission wavelength of 440 nm.

9. Calculation

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 value of standards and corresponding concentrations as y-axis and x-axis respectively. Create the standard curve (y = a1x + b1) with graph software (or Excel).

Sample calculations:

Animal tissue samples:

Definition: The amount of enzyme in 1 g tissue per 1 min that produces 1 μmol product at 37 °C is defined as 1 unit.

FAAH activity (U/kg wet weight) = (deltaF - b) / a / T x f / m x v x 9

Animal cell samples:

Definition: The amount of enzyme in 10^6 cells per 1 min that produces 1 μmol product at 37 °C is defined as 1 unit.

FAAH activity (U/10^6) = (deltaF - b) / a / T x f / n x v x 9

Where:

deltaF: Absolute fluorescence intensity of samples (F_{Total} enzyme well - F_{Non}-specific enzyme well)

T: Reaction time, 25 min

f: Dilution factor of sample before testing

m: Weight of tissue sample, kg

v: Volume of extraction solution, L

n: Number of cells, 10^6

9: Dilution factor of sample in the reaction system

10. Appendix I Performance Characteristics

Parameters:

Intra-assay Precision

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

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.01	0.05	0.1
%CV	4.9	6.0	3.7

Inter-assay Precision

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

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.01	0.05	0.1
%CV	12.2	0.2	7.5

Recovery

Three samples of high, medium, and low concentrations were tested in parallel 6 times each to obtain an average recovery rate of 103%.

Recovery

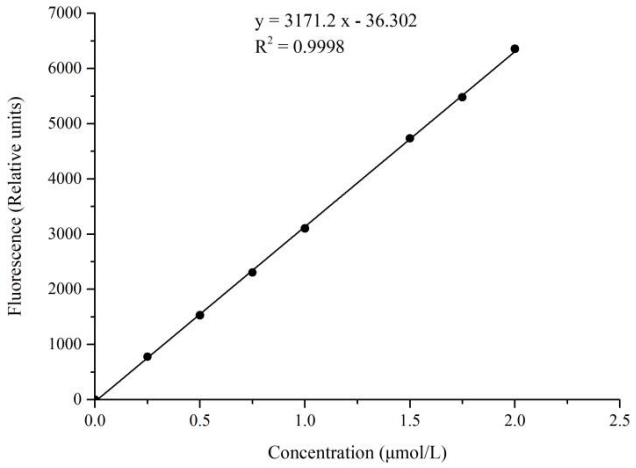
Parameters	Standard 1	Standard 2	Standard 3
Expected Conc. (U/L)	0.01	0.05	0.1
Observed Conc. (U/L)	0.011	0.051	0.099
Recovery rate (%)	105	100	103

Sensitivity

The analytical sensitivity of the assay is 0.004 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.

Standard curve data

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



Standard curve data

Concentration (µmol/L)	Fluorescence value 1	Fluorescence value 2	Average fluorescence value	Absolute fluorescence value
0	30	30	30	0
0.25	798	816	807	777
0.5	1519	1601	1560	1530
0.75	2350	2319	2335	2305
1	3162	3106	3134	3104
1.5	4768	4762	4765	4735
1.75	5433	5585	5509	5479

Concentration (μmol/L)	Fluorescence value 1	Fluorescence value 2	Average fluorescence value	Absolute fluorescence value
2	6352	6421	6387	6357

11. Appendix II Example Analysis

Example analysis:

Take 20 μL of 10% mouse liver tissue into the enzyme well and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 3171.2x - 36.302

Fluorescence value of enzyme well: 3099

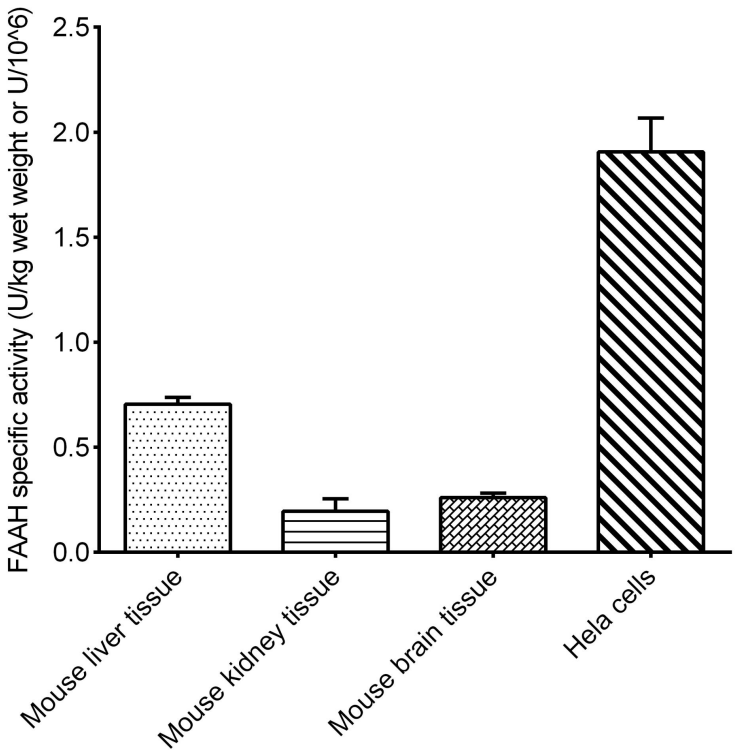
Fluorescence value of non-enzyme well: 2445

deltaF = F_{enzyme} - F_{non}-enzyme = 3099 - 2445 = 654

Calculation result:

FAAH activity (U/kg wet weight) = (654 + 36.302) / 3171.2 / 25 / 0.001 x 0.009 x 9 = 0.705 U/kg wet weight

Detection of 10% mouse liver tissue homogenate, 10% mouse kidney tissue homogenate, 10% mouse brain tissue homogenate, and 1 x 10^6 HeLa cells according to the protocol yielded the following results:



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.