（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-F082

Specification：96T(40 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=340 nm/440 nm)

Detection range：0.004-0.105 U/L

Elabscience® Fatty Acid Amide Hydrolase (FAAH) Activity Fluorometric Assay Kit

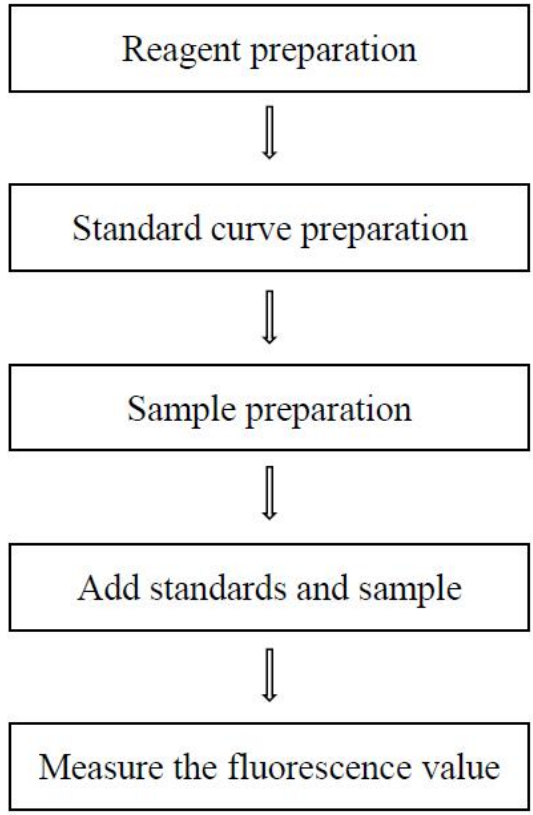
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary



Intended use

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

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-arachidenol glycerol in AEA and certain tissues.

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

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	55 mL × 1 vial	-20℃, 12 months, shading light
Reagent 2	Substrate	0.16 mL × 1 vial	-20℃, 12 months, shading light
Reagent 3	Inhibitor	0.08 mL × 1 vial	-20℃, 12 months, shading light
Reagent 4	1 mmol/L Standard Solution	0.4 mL × 1 vial	-20℃, 12 months, shading light
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.

For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② The preparation of substrate working solution:

Before testing, please prepare sufficient substrate working solution according to the test wells. For example, prepare 500 μL of substrate working solution (mix well 5 μL of substrate, 495 μL of buffer solution).The substrate working solution should be prepared on spot and used up within 1 day.

③ The preparation of inhibitor working solution:

Before testing, please prepare sufficient inhibitor working solution according to the test wells. For example, prepare 1000 μL of inhibitor working solution (mix well 5 μL of inhibitor, 995 μL of buffer solution). Store at -20℃ and used up within 2 days.

④ The preparation of 2 μmol/L standard solution:

Before testing, please prepare sufficient 2 μmol/L standard solution according to the test wells. For example, prepare 2500 μL of 2 μmol/L standard solution (mix well 5 μL of 1 mmol/L standard solution, 2495 μL of buffer solution).The 2 mmol/L standard solution should be prepared on spot and used up within 1 day.

⑤ The 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 a serial concentration. The recommended dilution gradient is as follows: 0, 0.25, 0.5, 0.75, 1, 1.5, 1.75, 2 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**0.25**	**0.5**	**0.75**	**1**	**1.5**	**1.75**	**2**
2 μmol/L standard (μL)	0	50	100	150	200	300	350	400
Buffer solution (μL)	400	350	300	250	200	100	50	0

Sample preparation

① 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 buffer solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

Cell (adherent or suspension) samples:

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

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

③ Homogenize 1×10^6 cells in 200 μL buffer solution with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

② **Dilution of sample**

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

Sample type	Dilution factor
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse brain tissue homogenate	1
10% Mouse lung tissue homogenate	1
1×10^6 293T cells	1

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

Operating steps

① Standard well: take 180 μL of standard with different concentrations and add it into corresponding enzyme-labeled well respectively.

Total enzyme well: take 20 μL of sample into the wells.

Nonspecfic enzyme well: take 20 μL of sample into the wells.

② Enzyme well: add 30 μL of buffer solution to the wells.

Non- enzyme tube: add 30 μL of inhibitor working solution to the well.

③ Incubate at 25℃ for 5 min protected from light.

④ Add 130 μL substrate working solution to the enzyme wells and non-enzyme wells.

⑤ Mix fully with microplate reader for 5 s and incubate at 37℃ for 25 min protected from light, measure the fluorescence intensity at the excitation wavelength of 340 nm and the emission wavelength of 440 nm.

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 absoluted fluorescence value.

3. Plot the standard curve by using absoluted fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve ($\mathbf{y = a_1x + b_1}$) with graph software (or EXCEL).

The sample:

Animal tissue samples:

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

$$\text{FAAH activity (U/kg wet weight)} = (\Delta F - b) \div a \div T \times f \div m \times v \times 9^*$$

Animal cell samples:

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

$$\text{FAAH activity (U/10^6)} = (\Delta F - b) \div a \div T \times f \div n \times v \times 9^*$$

[Note]

ΔF: Absolute fluorescence intensity of samples ($F_{\text{Total enzyme well}} - F_{\text{Nonspecfic enzyme well}}$).

T: Reaction time, 25 min.

f: Dilution factor of sample before tested.

m: the weight of tissue sample, kg.

v: the volume of the extraction solution, L.

n: the number of cells, 10^6.

9*: Dilution factor of sample in the reaction system.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

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.

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

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 103%.

	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

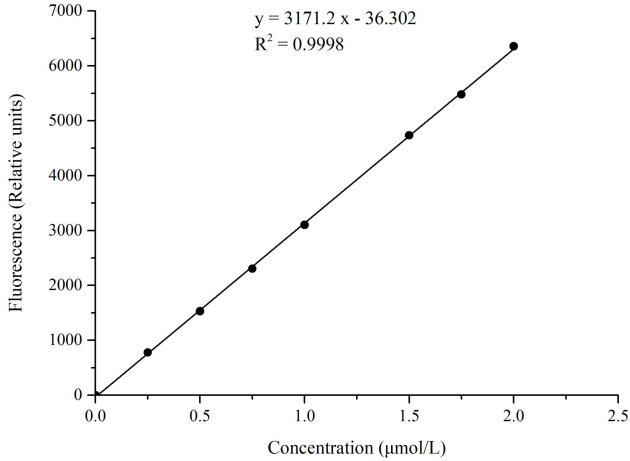
Sensitivity

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.

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

Concentration (μmol/L)	**0**	**0.25**	**0.5**	**0.75**	**1**	**1.5**	**1.75**	**2**
Fluorescence value	30	798	1519	2350	3162	4768	5433	6352
	30	816	1601	2319	3106	4762	5585	6421
Average fluorescence value	30	807	1560	2335	3134	4765	5509	6387
Absoluted fluorescence value	0	777	1530	2305	3104	4735	5479	6357



Appendix II Example Analysis

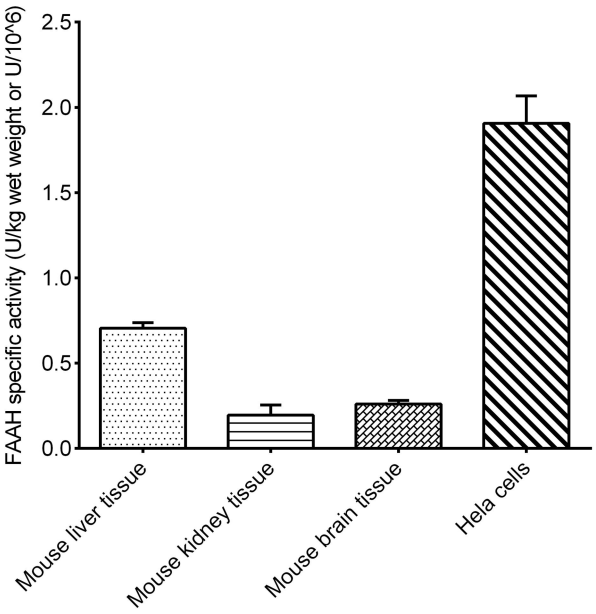
Example analysis：

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

Standard curve: y = 3171.2 x – 36.302, the fluorescence value of the enzyme is 3099, the fluorescence value of non-enzyme is 2445, $\Delta F = F_{enzyme} - F_{non\text{-}enzyme}$ = 3099 – 2445 = 654, the calculation result is:

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

Detect 10% mouse liver tissue homogenate, 10% mouse kidney tissue homogenate, 10% mouse brain tissue homogenate and 1×10^6 Hela cells according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.