Fatty Acid Amide Hydrolase Inhibitor Screening Assay Kit

Item No. 10005196

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -80°C kit. After opening the kit, store individual components as stated below.

Item Number	Item	Quantity/Size	Storage
700301	FAAH Assay Buffer (10X)	1 vial/5 ml	-20°C
700302	FAAH (human recombinant)	2 vials/120 µl	-80°C
700303	FAAH Substrate	1 vial/1.5 ml	-80°C
700307	JZL 195 Inhibitor Assay Reagent	1 vial	-20°C
400017	96-Well Solid Plate (black)	1 plate	RT
400012	96-Well Cover Sheet	1 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader with the ability to measure fluorescence with excitation and emission wavelengths of 340-360 and 450-465 nm, respectively
2. Adjustable pipettes and a repeating pipettor
3. A source of pure water; glass-distilled water or deionized water is acceptable *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*

INTRODUCTION

Background

The endocannabinoid system is an endogenous ubiquitous lipid signaling system involved in various regulatory functions throughout the body.[1-4] Arachidonoyl ethanolamide (AEA) and 2-arachidonoyl glycerol (2-AG) are two primary endocannabinoids that bind to G protein-coupled receptors, including the cannabinoid receptors CB_1 and CB_2, which have roles in motor control, cognition, emotional responses, and homeostasis and regulation of the autonomic nervous system, microcirculation, and the immune system, respectively. Endocannabinoids are released upon demand from lipid precursors in a receptor-dependent manner, transported into cells, and degraded primarily by two enzymes, monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH).[4]

FAAH is a serine hydrolase with a major role in the hydrolysis of endocannabinoids.[5-7] FAAH is localized to the cytosol and mitochondrial membranes and is highly expressed in the CNS but can also be found in peripheral tissues such as lung, gastrointestinal tract, kidney, liver, bladder, prostate, and testis.[6,8] It primarily catalyzes the inactivation of AEA *via* hydrolysis to arachidonic acid and ethanolamine but has broad substrate selectivity towards fatty acid amides, including oleamide, N-acyltaurines, and other N-acylethanolamines.[6] Genetic or pharmacologic knockdown of FAAH increases levels of AEA and dampens pain sensitivities and inflammatory endpoints in rodent models of inflammatory pain, allergic contact dermatitis, inflammatory bowel disease, and neuropathic pain, indicating therapeutic utility in the treatment of various conditions.[9]

About This Assay

Cayman's FAAH Inhibitor Screening Assay Kit provides a convenient fluorescence-based method for screening FAAH inhibitors. FAAH hydrolyzes AMC arachidonoyl amide resulting in the release of the fluorescent product, 7-amino-4-methylcoumarin (AMC). The fluorophore can be easily analyzed using an excitation wavelength of 340-360 nm and an emission wavelength of 450-465 nm.

PRE-ASSAY PREPARATION

Sample Preparation

All inhibitors, be they small molecules, natural products, or proteins, should be prepared in diluted FAAH Assay Buffer 1X or organic solvent at a concentration of 20X the desired final assay concentration (*e.g.*, for 10 µM final assay concentration, a 200 µM stock should be made). This solution may contain up to 100% DMSO or short-chain alcohols (*e.g.*, MeOH, EtOH). Dimethyl formamide (DMF) is not recommended as a solvent. The final concentration of organic solvents in the assay will then be 10% (see 'Effects of Solvents' on page 18).

Reagent Preparation

1. **FAAH Assay Buffer (1X) - (Item No. 700301)**

   Dilute 3 ml of FAAH Assay Buffer (10X) with 27 ml of pure water to make 30 ml FAAH Assay Buffer (1X). The FAAH Assay Buffer (1X) (125 mM Tris-HCl, pH 9.0, containing 1 mM EDTA) should be used in the assay and for diluting FAAH. FAAH Assay Buffer (1X) may be stored at 4°C for at least six months.

2. **FAAH (human recombinant) - (Item No. 700302)**

   Each vial contains 120 µl of recombinant human FAAH. Thaw the enzyme on ice, add 480 µl of FAAH Assay Buffer (1X) to the vial, and vortex. The diluted enzyme is stable for four hours on ice. One vial of enzyme diluted with FAAH Assay Buffer (1X) makes a sufficient volume of FAAH to assay 60 wells. Use the additional vial if assaying the entire plate.

3. **FAAH Substrate - (Item No. 700303)**

   The vial contains 1.5 ml of 400 µM AMC arachidonoyl amide. Dilute 50 µl of FAAH Substrate with 950 µl of ethanol. This is a sufficient volume for 96 wells. The addition of 10 µl to the assay yields a final concentration of 1 µM.

4. **JZL 195 Inhibitor Assay Reagent - (Item No. 700307)**

   The vial contains a lyophilized residue of JZL 195, a known inhibitor of FAAH. Dissolve the contents of the vial in 1 ml of solvent to produce a 20 µM solution. Suggested solvents include DMSO, methanol, or ethanol. DMF is not recommended. The reagent can be used to prepare a dose-response curve if desired. It is suggested to prepare half-log dilutions of the inhibitor to create a dose-response curve. Once dissolved, the reagent will be stable for three months when stored at -20°C.

ASSAY PROTOCOL

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. There is no specific pattern for using the wells on the plate. However, it is necessary to have three wells designated as 100% initial activity and three wells designated as background. It is suggested that each inhibitor, including the positive control JZL 195 Inhibitor Assay Reagent, be assayed in triplicate. It is suggested that the contents of each well be recorded on the template sheet provided on page 21. A typical layout of samples to be measured in triplicate is shown below in Figure 1.

	1	2	3	4	5	6	7	8	9	10	11	12
A	BW	BW	BW	7	7	7	15	15	15	23	23	23
B	A	A	A	8	8	8	16	16	16	24	24	24
C	1	1	1	9	9	9	17	17	17	25	25	25
D	2	2	2	10	10	10	18	18	18	26	26	26
E	3	3	3	11	11	11	19	19	19	27	27	27
F	4	4	4	12	12	12	20	20	20	28	28	28
G	5	5	5	13	13	13	21	21	21	29	29	29
H	6	6	6	14	14	14	22	22	22	30	30	30

BW - Background Wells
A - 100% Initial Activity Wells
1-30 - Inhibitor Wells

Figure 1. Sample plate format

Pipetting Hints

- It is recommended that an adjustable pipette be used to deliver reagents to the wells.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 200 µl in all the wells.
- Use the diluted assay buffer in the assay.
- All reagents should be prepared as described above. The FAAH (human, recombinant) enzyme should be kept on ice and all other reagents should be kept at room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended to assay the samples in triplicate, but it is at the user's discretion to do so.
- 30 inhibitor samples can be assayed in triplicate or 46 in duplicate.
- The assay is performed at 37°C.
- Monitor the fluorescence with an excitation wavelength of 340-360 nm and an emission wavelength of 450-465 nm.

Performing the Assay

1. **100% Initial Activity Wells** - add 170 µl of FAAH Assay Buffer (1X), 10 µl of diluted FAAH, and 10 µl of solvent (the same solvent used to dissolve the inhibitor) to three wells.
2. **Background Wells** - add 180 µl of FAAH Assay Buffer (1X) and 10 µl of solvent (the same solvent used to dissolve the inhibitor) to three wells.
3. **Inhibitor Wells** - add 170 µl of FAAH Assay Buffer (1X), 10 µl of diluted FAAH, and 10 µl of inhibitor to three wells.
4. Incubate for five minutes at 37°C.
5. Initiate the reactions by adding 10 µl of FAAH Substrate to all wells being used.
6. Cover the plate with the 96-Well Cover Sheet (Item No. 400012) and incubate for 30 minutes at 37°C.
7. Remove the plate cover and read the plate using an excitation wavelength of 340-360 nm and an emission wavelength of 450-465 nm. It may be necessary to adjust the gain setting on the instrument to allow for the measurement of all the samples.*

*If desired, the assay may be read kinetically rather than as an endpoint. Reading the assay kinetically may increase signal-to-background. The fluorescence should be measured at least once per minute at 37°C for 30 minutes. Determine the initial rate based on the linear portion of the kinetic curve. Calculations can be performed as shown on page 13 substituting initial rates for average fluorescence.

ANALYSIS

Calculations

1. Determine the average fluorescence (AF) of each sample.
2. Subtract the AF of the background wells from the AF of the 100% initial activity and inhibitor wells. These are the corrected values.
3. Determine the percent inhibition or percent activity for each inhibitor using one of the following equations:

$$\text{\% inhibition} = \left[\frac{\text{(corrected 100\% initial activity - corrected inhibitor activity)}}{\text{corrected 100\% initial activity}}\right] \times 100$$

$$\text{\% activity} = \left[\frac{\text{(corrected inhibitor activity)}}{\text{corrected 100\% initial activity}}\right] \times 100$$

4. Graph the percent inhibition or percent activity as a function of inhibitor concentration to determine the IC_{50} value (the concentration at which there is 50% inhibition) of the inhibitor. Inhibition of FAAH (human recombinant) enzyme by JZL 195 Inhibitor Assay Reagent is shown in Figure 3 (see page 17).

Performance Characteristics

Z´ Factor:

Z´ factor is a term used to describe the robustness of an assay, which is calculated using the equation below.[10]

$$Z' = 1 - \frac{3\sigma_{c+} + 3\sigma_{c-}}{|\mu_{c+} - \mu_{c-}|}$$

Where σ: Standard deviation
μ: Mean
c+: Positive control
c-: Negative control

The theoretical upper limit for the Z´ factor is 1.0. A robust assay has a Z´ factor >0.5. The Z´ factor for Cayman's FAAH Inhibitor Screening Assay Kit was determined to be 0.76.

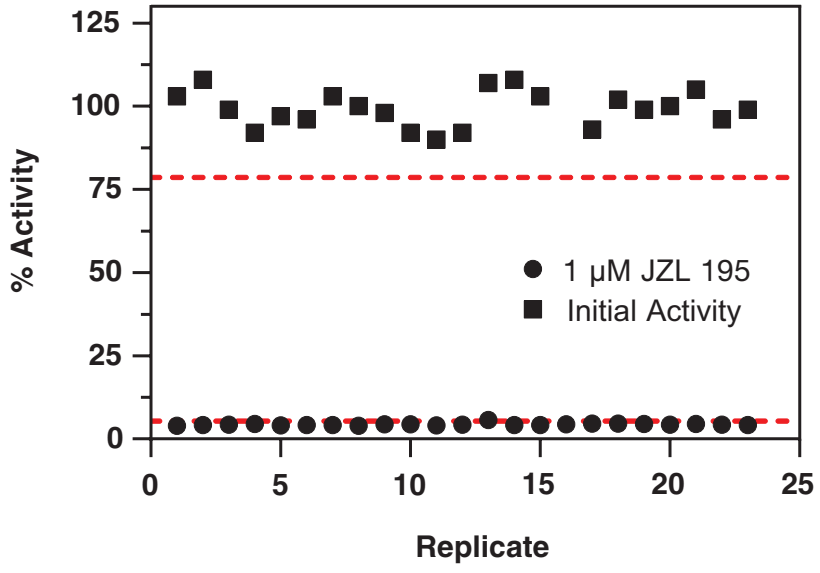

Figure 2. Typical Z' data for the FAAH Inhibitor Screening Kit. Data are shown from 24 replicates each for vehicel control ('Veh.') and 1 µM JZL 195 Inhibitor Assay Reagent prepared as described in the kit booklet. The calculated Z' factor for this experiment was 0.76. The red lines correspond to three standard deviations from the mean for each control value.

Precision:

When a series of sixteen FAAH measurements were performed on the same day, the intra-assay coefficient of variation was 2.9%. When a series of sixteen FAAH measurements were performed on five different days under the same experimental conditions, the inter-assay coefficient of variation was 3.1%.

Sample Data:

The data shown here is an example of the data typically produced with this kit; however, your results will not be identical to these. Do not use the data below to directly compare to your samples. Your results could differ substantially.

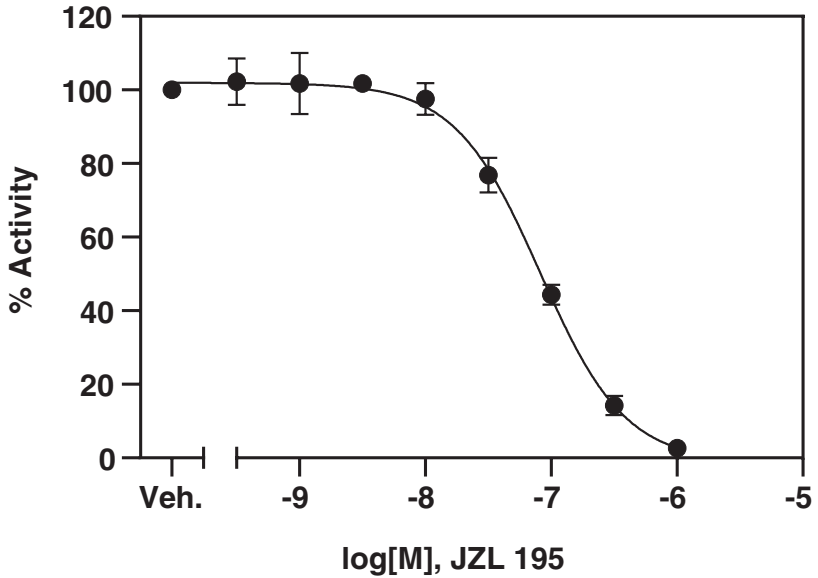

Figure 3. Inhibition of FAAH (human recombinant) by JZL 195 Inhibitor Assay Reagent. Data are plotted as the mean of triplicate measurements ± the standard deviation. "Veh." represents compound vehicle control.

Effects of Solvents:

Compounds may be prepared in organic solvents such as DMSO or short-chain alcohols (*e.g.*, MeOH, EtOH). A titration of organic solvents showed no change in signal up to 10% of the final organic concentration in the assay (substrate dilution accounts for 5%). DMF is not recommended. A proper vehicle control should be included in the assay.

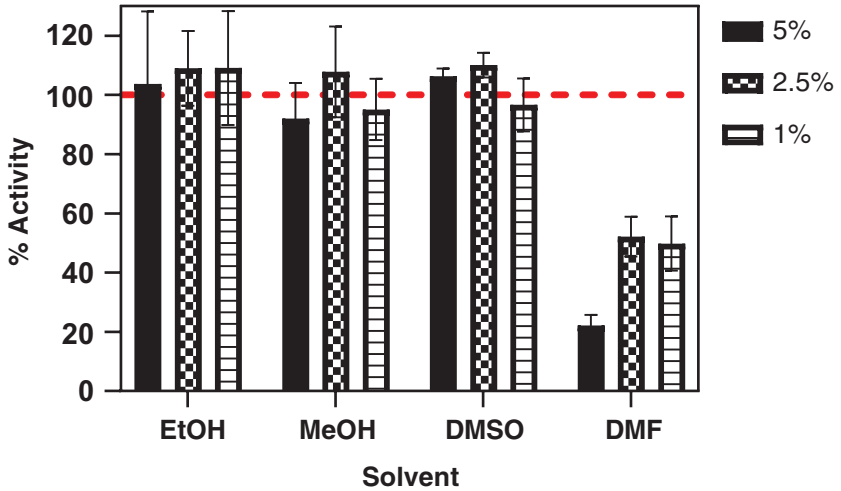

Figure 4. The effect of solvent on the readout of FAAH activity. The data are shown as the mean ± standard deviation for triplicate reactions containing the indicated concentration of solvents.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No fluorescence detected above background in any of the wells	A. Enzyme or substrate was not added to the wells B. Inhibitor concentration is too high and inhibited all of the enzyme activity	A. Make sure to add all the components to the well(s) and re-assay B. Reduce the inhibitor concentration and re-assay
The fluorometer exhibited 'MAX' values for the wells	The *gain* setting is too high	Reduce the *gain* and re-read
No inhibition seen with compound	A. The compound concentration is not high enough B. The compound is not an inhibitor of the enzyme	Increase the compound concentration and re-assay

References

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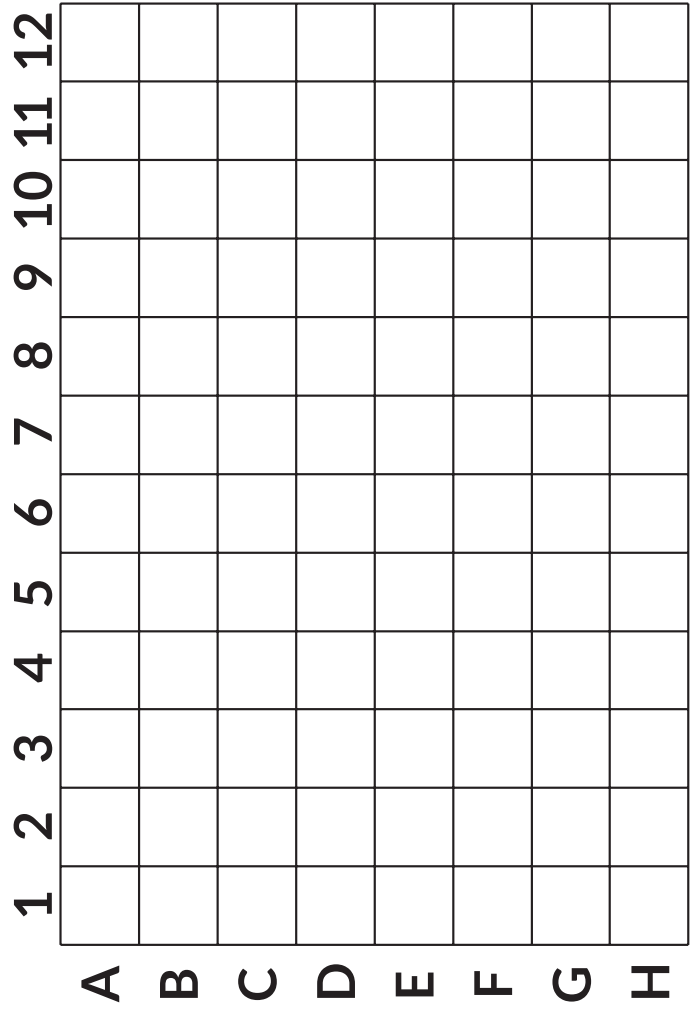

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.