

FAAH Inhibitor Screening Assay Kit

Catalog No. 10005196

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GENERAL INFORMATION

Materials Supplied

Catalog Number	Item	Quantity
700301	FAAH Assay Buffer (10X)	1 vial
700302	FAAH (human recombinant)	2 vials
700303	FAAH Substrate	1 vial
400017	96-Well Plate (black)	1 plate
400012	96-Well Cover Sheet	1 cover

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.



WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

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

Fax: 734-971-3641

E-Mail: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

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 at -80°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A fluorometer with the capacity to measure fluorescence using an excitation wavelength of 340-360 nm and an emission wavelength of 450-465 nm
2. Adjustable pipettes and a repeat pipettor
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

INTRODUCTION

Background

The endocannabinoid system is a ubiquitous lipid signaling system that is involved in various regulatory functions throughout the body. The main endocannabinoids are arachidonoyl ethanolamide (AEA) and 2-arachidonoyl glycerol (2-AG). They bind to G protein-coupled receptors, of which the cannabinoid (CB₁) receptors is densely distributed in areas of the brain related to motor control, cognition, emotional responses, and homeostasis.¹⁻⁴ Acting *via* the CB₂ receptor in the peripheral tissues, the endocannabinoid system is one of the crucial modulators of the autonomic nervous system, the immune system, and microcirculation. Endocannabinoids are released upon demand from lipid precursors in a receptor-dependent manner. They are transported into cells by an apparently specific uptake system and degraded primarily by two enzymes, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGL, MAGL) resulting in the termination of their biological actions.⁵ FAAH, a serine hydrolase, can degrade many fatty acid amides, including AEA. Although FAAH can hydrolyze 2-AG, the main enzyme responsible for the inactivation of this monoacylglyceride is another serine hydrolase, MAGL. Finding inhibitors to these endocannabinoid hydrolases could offer another approach in the treatment of pain, obesity, and various neurological diseases, where higher endocannabinoid activity would be beneficial. An advantage of such enzyme inhibition over direct cannabinoid agonists could result in higher selectivity, as it would increase activity of the endocannabinoid system only at sites where on-going production of endocannabinoids is taking place.⁶

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.

Reagent Preparation

1. Assay Buffer (10X) - (Catalog No. 700301)

Dilute 3 ml of Assay Buffer concentrate with 27 ml of HPLC-grade water. This final Buffer (125 mM Tris-HCl, pH 9.0, containing 1 mM EDTA) should be used in the assay and for diluting FAAH. When stored at 4°C, this diluted buffer is stable for at least six months.

2. FAAH (human recombinant) - (Catalog No. 700302)

Each vial contains 120 µl of human recombinant FAAH. Thaw the enzyme on ice, add 480 µl of diluted assay buffer to the vial, and vortex. The diluted enzyme is stable for four hours on ice. One vial of enzyme is enough FAAH to assay 60 wells. Use the additional vial if assaying the entire plate.

3. FAAH Substrate - (Catalog No. 700303)

The vial contains 1.5 ml of 400 µM AMC Arachidonoyl amide. It is ready to use in the assay. The addition of 10 µl to the assay yields a final concentration of 20 µM. *NOTE: The substrate concentration may be reduced with ethanol at the user's discretion.*

Plate Set Up

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 wells. We suggest that each inhibitor sample be assayed in triplicate and that you record the contents of each well on the template sheet provided on page 15. A typical layout of samples and compounds 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.
- It is not necessary to use all the wells on the plate at one time.
- If the appropriate inhibitor concentration is not known, it may be necessary to assay at several concentrations.
- We recommend assaying samples in triplicate, but it is the user's discretion to do so.
- Thirty inhibitor samples can be assayed in triplicate or forty-six in duplicate.
- The assay temperature is 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 assay buffer, 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 assay buffer and 10 μl of solvent (the same solvent used to dissolve the inhibitor) to three wells.
3. **Inhibitor Wells** - add 170 μl of assay buffer, 10 μl of diluted FAAH, and 10 μl of inhibitor* to three wells.
4. Initiate the reactions by adding 10 μl of substrate to all the wells being used.
5. Cover the plate with the plate cover and incubate for 30 minutes at 37°C.
6. 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.

*Inhibitors can be dissolved in assay buffer, ethanol, methanol, or dimethylsulfoxide and should be added to the assay in a final volume of 10 μl . In the event that the appropriate concentration of inhibitor needed for FAAH inhibition is completely unknown, we recommend that several concentrations of the inhibitor be assayed.

Calculations

1. Determine the average fluorescence of each sample.
2. Subtract the fluorescence of the background wells from the fluorescence of the 100% initial activity and the inhibitor wells.
3. Determine the percent inhibition for each sample. To do this, subtract each inhibitor sample value from the 100% initial activity sample value. Divide the result by the 100% initial activity value and then multiply by 100 to give the percent inhibition.
4. Either graph the Percent Inhibition or Percent Initial Activity as a function of the inhibitor concentration to determine the IC_{50} value (concentration at which there was 50% inhibition). An example of FAAH inhibition by CAY10435, a specific FAAH inhibitor, is shown in Figure 2, on page 11.⁷

$$\% \text{Inhibition} = \left[\frac{(\text{Initial Activity} - \text{Sample})}{\text{Initial Activity}} \right] \times 100$$

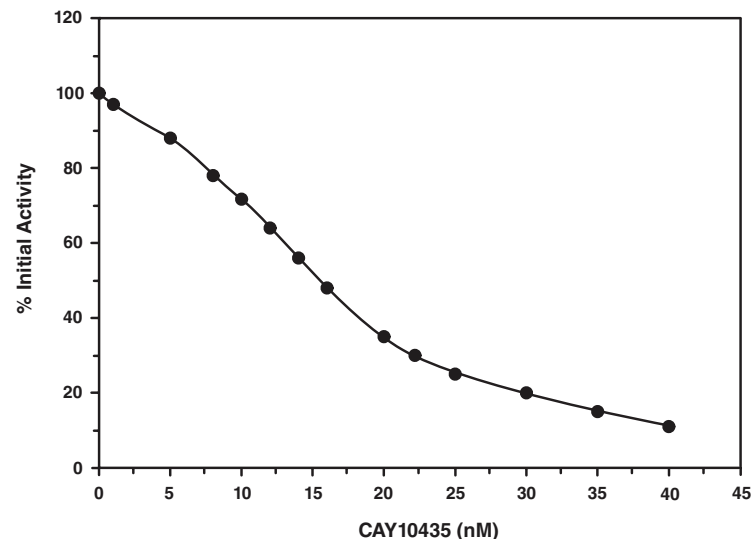


Figure 2. Inhibition of FAAH by CAY10435 ($IC_{50} = 15 \text{ nM}$)

Performance Characteristics

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%.

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Carefully tap the side of the plate with your finger to remove bubbles B. Be careful not to splash the contents of the wells
No fluorescence detected above background in any of the wells	A. Either substrate or FAAH was not added to the wells B. The inhibitor concentration was too high	A. Make sure to add all the components to the wells 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

1. Stella, N., Schweitzer, P., and Piomelli, D. A second endogenous cannabinoid that modulates long-term potentiation. *Nature* **388**, 773-778 (1997).
2. Sugiura, T., Kodaka, T., Nakane, S., *et al.* Evidence that the cannabinoid CB₁ receptor is a 2-arachidonoylglycerol receptor. Structure-activity relationship of 2-arachidonoylglycerol, ether-linked analogues, and related compounds. *J. Biol. Chem.* **274**, 2794-2801 (1999).

3. Kondo, S., Kondo, H., Nakane, S., *et al.* 2-Arachidonoylglycerol, an endogenous cannabinoid receptor agonist: Identification as one of the major species of monoacylglycerols in various rat tissues, and evidence for its generation through Ca²⁺-dependent and -independent mechanisms. *FEBS Lett.* **429**, 152-156 (1998).
4. Rodríguez De Fonseca, F., Del Arco, I., Bermudez-Silva, F., *et al.* The endocannabinoid system: Physiology and pharmacology. *Alcohol & Alcoholism* **40(1)**, 2-14 (2005).
5. Dinh, T.P., Carpenter, D., Leslie, F.M., *et al.* Brain monoacylglyceride lipase participating in endocannabinoid inactivation. *Proc. Natl. Acad. Sci. USA* **99(16)**, 10819-10824 (2002).
6. Lambert, D.M. and Fowler, C.J. The endocannabinoid system: Drug targets, lead compounds, and potential therapeutic applications. *J. Med. Chem.* **48(16)**, 5059-5087 (2005).
7. Boger, D.L., Sato, H., Lerner, A.E., *et al.* Exceptionally potent inhibitors of fatty acid amide hydrolase: The enzyme responsible for degradation of endogenous oleamide and anandamide. *Proc. Natl. Acad. Sci. USA* **97**, 5044-5049 (2000).

Related Products

AMC Arachidonoyl Amide - Cat. No. 10005098
 O-Arachidonoyl Glycidol - Cat. No. 10010547
 CAY10400 - Cat. No. 71650
 CAY10401 - Cat. No. 71652
 CAY10402 - Cat. No. 71655
 CAY10435 - Cat. No. 10005102
 CAY10570 - Cat. No. 10010032
 JP83 - Cat. No. 10008660
 JP104 - Cat. No. 10008661
 Monoacylglycerol Lipase Inhibitor Screening Assay Kit - Cat. No. 10005192
 Oleoyl Ethyl Amide - Cat. No. 10005459
 PF-750 - Cat. No. 10010908
 PF-622 - Cat. No. 10010907
 URB597 - Cat. No. 10046

Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

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NOTES

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