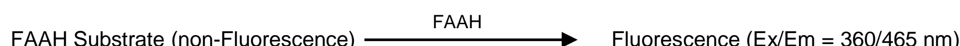
Fatty Acid Amide Hydrolase (FAAH) Activity Assay Kit (Fluorometric)

8/18

(Catalog # K434-100; 100 assays; Store at -20°C)

I. Introduction:

Fatty Acid Amide Hydrolase (FAAH; EC: 3.5.1.99, Oleamide Hydrolase, Anandamide Amidohydrolase) is a mammalian integral membrane enzyme, and is a key regulator in lipid signaling. FAAH degrades naturally occurring fatty acid amides such as cannabinoid anandamide and oleamide, a sleep-inducing substance. In general, fatty acid amides are endogenous lipid ligands which activate the cannabinoid (CB) G-protein coupled receptors CB1 and CB2. CB1 and CB2 modulate physiological and behavioral processes such as pain, and anti-inflammation. Therefore, FAAH is crucial in the termination of fatty acid amide bioactive functions. Recent studies showed that blockage of FAAH activity led to increased fatty acid amides levels in nervous system and peripheral tissues. Thus, the study of FAAH and its potential inhibitors could develop novel treatment strategies for pain and central nerve system disorders due to the analgesic, anxiolytic and anti-inflammatory properties that are observed when elevated fatty acid amides are present in humans. In BioVision's FAAH Assay Kit, FAAH hydrolyzes a non-fluorescent substrate releasing 7-amino-4-methylcoumarin (AMC), a fluorophore, which can be easily measured at Ex/Em= 360/465 nm. The kit provides a specific inhibitor that can be used to compensate for potential non-specfic background in unknown samples. The stable fluorescence signal is positively correlated to FAAH enzymatic activity in samples. The kit offers a rapid, simple, sensitive, reproducible assay and is suitable for detecting FAAH activity as low as 0.1 µU.



II. Application:

- Measurement of FAAH activity of pure enzyme, various tissues/cells.
- Study of Fatty Acid Amide and Fatty Acid Amide Hydrolase signaling systems in various cell types.

III. Sample Type:

- Animal tissues: liver and brain, etc.
- Cell culture: adherent or suspension cells

IV. Kit Contents:

Components	K434-100	Cap Code	Part Number
FAAH Assay Buffer	25 ml	WM	K434-100-1
FAAH Substrate (in DMSO)	100 µl	Amber	K434-100-2
FAAH Positive Control	40 µl	Red	K434-100-3
AMC Standard (1 mM)	100 µl	Yellow	K434-100-4
FAAH Inhibitor (in DMSO)	100 µl	Blue	K434-100-5

V. User Supplied Reagents and Equipment:

- 96-well white opaque plate with flat bottom.
- Multi-well spectrophotometer (fluorescence plate reader)
- Multi-channel pipette

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly spin small vials prior to opening. Read entire protocol before performing the assay. Unless specified, bring assay components to room temperature (RT) before use.

- **FAAH Substrate (in DMSO), AMC Standard (1 mM) and FAAH Inhibitor (in DMSO):** Thaw these vials at RT and mix well. Store at -20 °C. Avoid repeated freeze/thaw. Use within two months.
- **FAAH Positive Control:** Aliquot and store at -70 °C. Avoid repeated freeze/thaw. Use within two months. Keep on ice while in use.

VII. FAAH Activity Assay Protocol:

1. **Sample Preparation:** Homogenize tissue (~10 mg) or cells (1 x 10^6) with 100 µl ice-cold FAAH Assay Buffer. Keep on ice for 10 min. Centrifuge at 10,000 x *g*, 4 °C for 5 min. and collect supernatant. **Isolated Microsome:** follow standard protocols to isolate microsomes; however, we recommend BioVision Cat. # K249-50 in order to obtain optimal yields. Dilute sample(s) 10-fold with FAAH assay buffer (i.e. 10 µl of sample into 90 µl of FAAH Assay Buffer). Add 2-50 µl into desired well(s) of a 96-well white plate. **FAAH Positive Control**: add 4-12 µl of FAAH Positive Control into desired well(s). Adjust the volume of Positive Control and sample wells to 50 µl/well with FAAH Assay Buffer.
   Notes:
   a. For unknown samples, we suggest doing pilot experiment and testing several amounts of sample to ensure the readings are within the Standard Curve range.
   b. If sample has high background, prepare duplicate sample well(s) as sample background control(s).
2. **AMC Standard Curve:** Dilute AMC Standard 100-fold to 10 µM (10 pmol/µl) by adding 10 µl of 1 mM AMC Standard to 990 µl of dH_2O. Add 0, 2, 4, 6, 8, and 10 µl of 10 µM AMC Standard into a series of wells in a 96-well plate to generate 0, 20, 40, 60, 80 and 100 pmol/well of AMC Standard. Adjust the volume to 100 µl/well with FAAH Assay Buffer.

3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing:

	Reaction Mix	*Background Control Mix
FAAH Assay Buffer	49 µl	48 µl
FAAH Substrate	1 µl	1 µl
FAAH Inhibitor	----	1 µl

Mix and add 50 µl of Reaction Mix into each well containing Positive Control, and Samples. Mix well.
* For samples having background, add 50 µl of Background Control Mix to sample background control well(s).

4. Measurement: Measure fluorescence (Ex/Em = 360/465 nm) immediately in kinetic mode for 10-60 min. at 37°C.
Note: Incubation time depends on the FAAH activity in the samples. We recommend measuring fluorescence in kinetic mode, and choosing two time points (t_1 and t_2) in the linear range to calculate the FAAH activity of the samples. The AMC Standard Curve can be read in endpoint mode (i.e. at the end of incubation time).

5. Calculation: Subtract 0 Standard reading from all readings. Plot the AMC Standard curve. Choose two time points (t_1 and t_2) within the linear portion of graph. If sample background control reading is significant, subtract the sample background control reading from sample reading. Calculate the FAAH activity of the test sample: $\Delta RFU = RFU_2 - RFU_1$. Apply ΔRFU to AMC Standard Curve to get B pmol of AMC generated by FAAH during the reaction time ($\Delta t = t_2 - t_1$).

Sample FAAH Activity = B/(Δt X V) x D = pmol/min/µl = µU/µl = mU/ml

Where: **B** is AMC amount in the sample well from Standard Curve (pmol)
Δt is reaction time (min.)
V is sample volume added into the reaction well (µl)
D is dilution factor (D=1 if undiluted)

FAAH Activity in samples can also be expressed in mU/mg of protein.
Unit Definition: One unit of FAAH is defined as the amount of enzyme that generates 1.0 µmol of AMC per min. at pH 8.8 at 37°C.

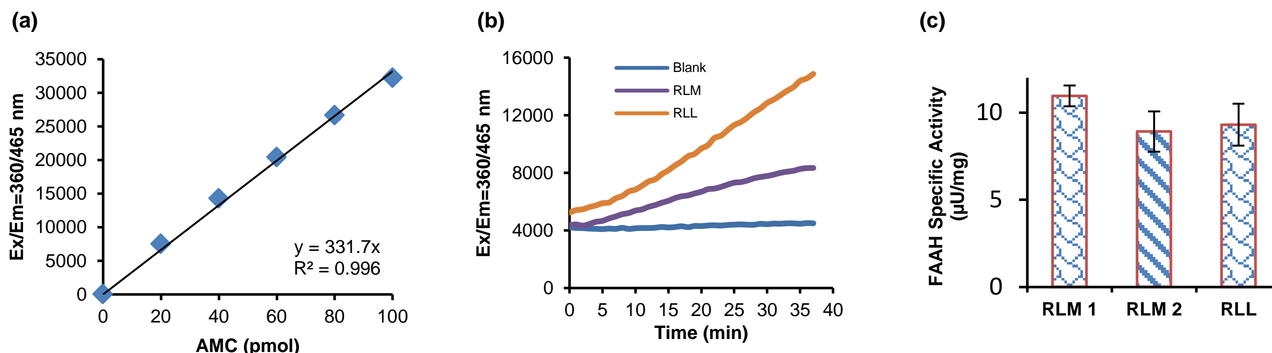


Figure: (a) AMC Standard Curve. (b) Kinetic measurement of FAAH Activity in lysates prepared from blank (no sample), rat liver microsome (RLM: 32 µg) and rat liver lysate (RLL: 80 µg) and FAAH Positive Control. (c) FAAH specific activity of two rat liver microsome preparations (RLM1 and RLM2) and rat liver lysate (RLL: 80 µg). Assays were performed following the kit protocol.

VIII. Related Products:

Monoacylglycerol Lipase (MAGL) Activity Assay Kit (K561)
Cyclooxygenase (COX) Activity Assay Kit (Fluorometric) (K549)
Peroxidase Activity Assay Kit (K772)
Myeloperoxidase (MPO) Colorimetric Activity Assay Kit (K744)
Myeloperoxidase (MPO) Inhibitor Screening Kit (K746)
Monoacylglycerol Lipase Inhibitor Screening Kit (K474)
COX-2 Inhibitor Screening Kit (K547)
Myeloperoxidase (MPO) Peroxidation Activity Assay Kit (K747)
Myeloperoxidase (MPO) Fluorometric Activity Assay Kit (K745)
15-PDGH Inhibitor Screening Assay (K503-100)

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