Technical Bulletin

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

Catalog Number MAK402

Product Description

Fatty Acid Amide Hydrolase (FAAH; Oleamide hydrolase, Anandamide amidohydrolase) is a mammalian integral membrane enzyme that 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 inflammation. Therefore, FAAH is crucial in the termination of fatty acid amide bioactive functions. Studies have shown 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 this assay, FAAH hydrolyzes a non-fluorescent substrate releasing 7-amino-4-methylcoumarin (AMC), a fluorophore, which can be easily measured at λ_{Ex} = 360 nm/λ_{Em} = 465 nm. The kit includes a specific inhibitor that can be used to compensate for potential non-specific background signal 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.

The kit is suitable for the measurement of FAAH activity of pure enzyme, in various tissues (e.g., liver and brain) and in adherent or suspension cells. The kit is also suitable for the study of Fatty Acid Amide and Fatty Acid Amide Hydrolase signaling systems in various cell types.

Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

- FAAH Assay Buffer 25 mL
  Catalog Number MAK402A
- FAAH Substrate (in DMSO) 100 µL
  Catalog Number MAK402B
- FAAH Positive Control 40 µL
  Catalog Number MAK402C
- AMC Standard (1 mM) 100 µL
  Catalog Number MAK402D
- FAAH Inhibitor (in DMSO) 100 µL
  Catalog Number MAK402E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Fluorescence multiwell plate reader
- White, opaque flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- Microsome Isolation Kit (Catalog Number MAK340)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Unless specified, bring assay components to room temperature prior to use.

FAAH Assay Buffer: Ready to use. Chill an appropriate amount of FAAH Assay Buffer for use in Sample Preparation.

FAAH Substrate (in DMSO), AMC Standard (1 mM) and FAAH Inhibitor (in DMSO): Thaw vials at room temperature and mix well. Store at -20 °C. Avoid repeated freeze/thaw cycles. Use within two months upon opening.

FAAH Positive Control: Aliquot and store at -70 °C. Avoid repeated freeze/thaw cycles. Use within two months upon opening. Keep on ice while in use.

Procedure

Tissue or Cell Sample Preparation

1. Homogenize tissue (~10 mg) or cells (1×10^6 cells) with 100 µL of ice-cold FAAH Assay Buffer. Keep on ice for 10 minutes.
2. Centrifuge at 10,000 × *g*, 4 °C for 5 minutes.
3. Collect supernatant.
4. Dilute sample(s) 10-fold with FAAH Assay Buffer (i.e., 10 µL of sample into 90 µL of FAAH Assay Buffer).
5. Add 2-50 µL into desired well(s) of a 96-well white plate. For unknown samples, test several doses to ensure the readings are within the Standard Curve range.
6. Adjust the total volume to 50 µL/well with FAAH Assay Buffer.

Isolated Microsome Preparation

1. Follow the Microsome Isolation Kit (Catalog Number MAK340) technical bulletin to isolate microsomes.
2. Dilute sample(s) 10-fold with FAAH Assay Buffer (i.e., 10 µL of sample into 90 µL of FAAH Assay Buffer).
3. Add 2-50 µL into desired well(s) of a 96-well white plate. For unknown samples, test several doses to ensure the readings are within the Standard Curve range.
4. Adjust the total volume to 50 µL/well with FAAH Assay Buffer.

FAAH Positive Control

Add 4-12 µL of FAAH Positive Control to designated well(s). Adjust the total volume to 50 µL/well with FAAH Assay Buffer.

Background Control

If sample has high background, prepare duplicate sample well(s) for use as Sample Background Control(s).

Standard Curve Preparation

Prepare a 10 µM (10 pmol/µL) AMC Standard by diluting 10 µL of AMC Standard (1 mM) with 990 µL of purified water. Prepare AMC Standards according to Table 1. Mix well.

Table 1.
Preparation of AMC Standards

Well	10 µM AMC Standard	FAAH Assay Buffer	AMC (pmol/ well)
1	0 µL	100 µL	0
2	2 µL	98 µL	20
3	4 µL	96 µL	40
4	6 µL	94 µL	60
5	8 µL	92 µL	80
6	10 µL	90 µL	100

Reaction Mix

1. Mix enough reagents for the number of assays to be performed.
   a. For each well containing Sample or Positive Control, prepare 50 µL of Reaction Mix according to Table 2, mix well.
   b. For each Sample Background Control well, prepare Background Control Mix according to Table 2, mix well. It is not necessary to prepare Background Control Mix if background controls were not required.

Table 2.
Reaction Mix Preparation

Reagent	Reaction Mix	Background Control Mix
FAAH Assay Buffer	49 µL	48 µL
FAAH Substrate	1 µL	1 µL
FAAH Inhibitor	-	1 µL

Assay Procedure

1. Add 50 µL of Reaction Mix into each well containing Positive Control and Samples. Mix well.
2. For samples requiring a background control, add 50 µL of Background Control Mix to sample background control well(s). Mix well.

Measurement

Immediately measure fluorescence at λ_{Ex} = 360 nm/λ_{Em} = 465 nm in kinetic mode for 10-60 minutes at 37 °C.

Incubation time will depend on the FAAH activity in the samples. The AMC Standard Curve can be read in endpoint mode at the end of the incubation.

Results

1. Subtract 0 Standard reading from all readings.
2. Plot the AMC Standard curve.
3. Choose two time points (T_1 and T_2) within the linear portion of graph.
4. If Sample Background Control reading is significant, subtract the Sample Background Control reading from the Sample reading.
5. Calculate the FAAH activity of the test sample: $\Delta RFU = RFU_2 - RFU_1$.
6. Apply ΔRFU to AMC Standard Curve to get B pmol of AMC generated by FAAH during the reaction time ($\Delta T = T_2 - T_1$).

FAAH Activity (pmol/min/μL = μU/μL = mU/mL) =

$$[B/(\Delta T \times V)] \times D$$

where

B = AMC amount in the sample well from the Standard Curve (pmol)

ΔT = Reaction time (minutes)

V = Sample volume added into the reaction well (μL)

D = 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 minute at pH 8.8 at 37 °C.

Figure 1.
Typical AMC Standard Curve

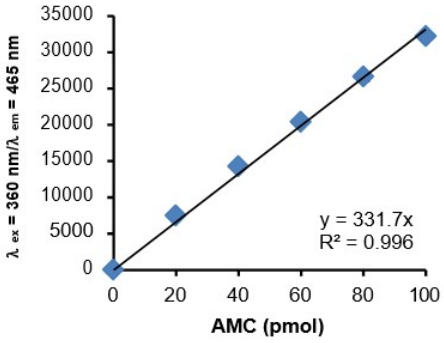


Figure 2.
Kinetic measurement of FAAH Activity in lysates prepared from blank (no sample), rat liver microsome (RLM: 32 μg) and rat liver lysate (RLL: 80 μg).

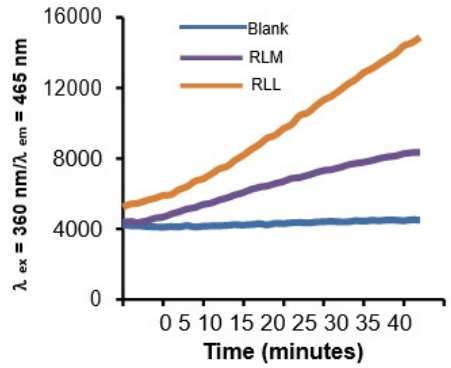
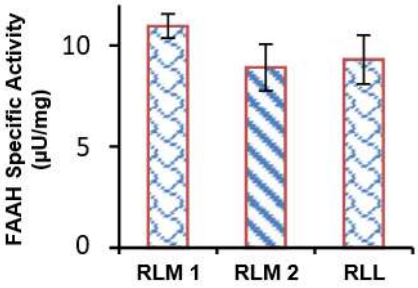


Figure 3.
FAAH specific activity of two rat liver microsome preparations (RLM1 and RLM2) and rat liver lysate (RLL). Assays were performed following the kit protocol.



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