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# ab252895 Fatty Acid Amide Hydrolase Activity Assay Kit (Fluorometric)

View Fatty Acid Amide Hydrolase Activity Assay Kit (Fluorometric) datasheet:

http://www.abcam.com/ab252895

[use http://www.abcam.cn/ab252895 for China, or http://www.abcam.co.jp/ab252895 for Japan)

For the measurement of FAAH activity of pure enzyme and of various tissues/cells.

This product is for research use only and is not intended for diagnostic use.

## Table of Contents

# 1. Overview

In the Fatty Acid Amide Hydrolase Activity Assay Kit (Fluorometric) (ab252895), 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-specific 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.

## 2. Protocol Summary

Prepare Samples, Standards, Positive Control and Background controls as directed.

Prepare Reaction Mix and Background Control Mix.

Add Samples and Positive Control (50 µL each) to Reaction Mix (50 µL). Add Background Control, Samples (50 µL) to Background Control Mix to 96-well, white, flat-bottomed plate. Add Standards (100 µL) to appropriate wells.

Measure fluorescence (Ex/Em = 360/465 nm) immediately in kinetic mode for 10-60 mins at 37°C. Standards may be read in end-point mode.

## 3. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components.

Avoid repeated freeze-thaws of reagents.

| Item | Quantity | Storage temperature (before prep) | Storage temperature (after prep) |
|---|---|---|---|
| AMC Standard (1 mM) | 100 µL | -20°C | -20°C |
| Assay Buffer LIX/FAAH Assay Buffer | 25 mL | -20°C | -20°C |
| FAAH Inhibitor (in DMSO) | 100 µL | -20°C | -20°C |
| FAAH Positive Control | 40 µL | -20°C | -20°C |
| FAAH Substrate (in DMSO) | 100 µL | -20°C | -20°C |

## 4. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Fluorescent microplate reader capable of measuring Ex/Em = 360/465 nm.
- 96-well white plate with flat bottom.
- Dounce homogenizer.

## 5. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

## 6. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

### 6.1 AMC Standard (1 mM)

Ready to use as supplied. Thaw vial at RT and mix well. Store at -20°C. Avoid repeated freeze/thaw. Use within two months.

### 6.2 Assay Buffer LIX/FAAH Assay Buffer

Ready to use as supplied.

### 6.3 FAAH Inhibitor (in DMSO)

Ready to use as supplied. Thaw vial at RT and mix well. Store at -20°C. Avoid repeated freeze/thaw. Use within two months.

### 6.4 FAAH Positive Control

Aliquot and store at -20°C. Avoid repeated freeze/thaw. Use within two months. Keep on ice while in use.

### 6.5 FAAH Substrate (in DMSO)

Ready to use as supplied. Thaw vial at RT and mix well. Store at -20°C. Avoid repeated freeze/thaw. Use within two months.

## 7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

**7.1** Dilute AMC Standard 100-fold to 10 µM (10 pmol/µL) by adding 10 µL of 1 mM AMC Standard to 990 µL of $dH_2O$.

**7.2** 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.

**7.3** Adjust the volume to 100 µL/well with Assay Buffer LIX/FAAH Assay Buffer.

| Standard# | 10 µM AMC Standard (µL) | Assay Buffer LIX/FAAH Assay Buffer (µL) | Final volume standard in well (µL) | End amount AMC in well (pmol/well) |
|---|---|---|---|---|
| **1** | 0 | 200 | 100 | 0 |
| **2** | 4 | 196 | 100 | 20 |
| **3** | 8 | 192 | 100 | 40 |
| **4** | 12 | 188 | 100 | 60 |
| **5** | 16 | 184 | 100 | 80 |
| **6** | 20 | 180 | 100 | 100 |

Each dilution has enough amount of standard to set up duplicate readings (2 x 100 µL).

## 8. Sample Preparation

**General sample information:**

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice and proceed with the Sample Preparation step. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

### 8.1 Tissue:

8.1.1 Homogenize tissue (~10 mg) or cells (1 x $10^6$) with 100 µL ice-cold Assay Buffer LIX/FAAH Assay Buffer. Keep on ice for 10 mins.

8.1.2 Centrifuge at 10,000 x *g*, 4°C for 5 mins and collect supernatant.

### 8.2 Isolated microsomes:

8.2.1 Follow standard protocols to isolate microsomes.

8.2.2 Dilute sample(s) 10-fold with Assay Buffer LIX/FAAH assay buffer (i.e. 10 µL of sample into 90 µL of Assay Buffer LIX/FAAH Assay Buffer).

8.2.3 Add 2-50 µL into desired well(s) of a 96-well white plate.

**Δ Note:** For unknown samples, we suggest doing pilot experiment and testing several amounts of sample to ensure the readings are within the Standard Curve range.

## 9. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

### 9.1 Reaction mix preparation:

1. Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µL Mix containing:

| Component | Reaction Mix (µL) | Background Control Mix (µL) |
|---|---|---|
| Assay Buffer LIX/FAAH Assay Buffer | 49 | 48 |
| FAAH Substrate | 1 | 1 |
| FAAH Inhibitor | --- | 1 |

2. Mix and add 50 µL of Reaction Mix into each well containing Positive Control, and Samples. Mix well.

**Δ Note:** For samples having background, add 50 µL of Background Control Mix to sample background control well(s).

3. 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 Assay Buffer LIX/FAAH Assay Buffer.

## 9.2 Measurement:

1. Measure fluorescence (Ex/Em = 360/465 nm) immediately in kinetic mode for 10-60 mins at 37°C.

**Δ Note:** Incubation time depends on the FAAH activity in the samples. We recommend measuring fluorescence in kinetic mode, and choosing two time points (t1 and t2) in the linear range to calculate the FAAH activity of the samples.

**Δ Note:** The AMC Standard Curve can be read in endpoint mode (i.e. at the end of incubation time).

## 9.3 Calculation:

1. Subtract 0 Standard reading from all readings.
2. Plot the AMC Standard curve.
3. Choose two time points (t1 and t2) within the linear portion of graph.
4. If sample background control reading is significant, subtract the sample background control reading from sample reading.
5. Calculate the FAAH activity of the test sample: ΔRFU = RFU2 – RFU1.
6. Apply ΔRFU to AMC Standard Curve to get B pmol of AMC generated by FAAH during the reaction time (Δt = t2 - t1).

**FAAH Activity = B/(Δt x V) x D**

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

## 10. Typical Data

Data provided **for demonstration purposes only**.

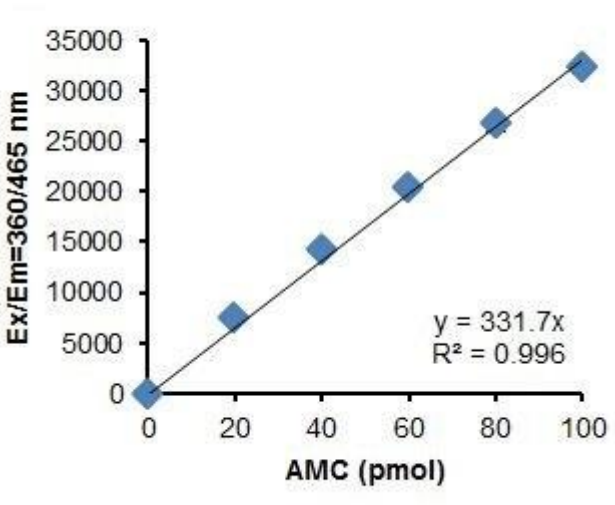


**Figure 1**. AMC standard curve.

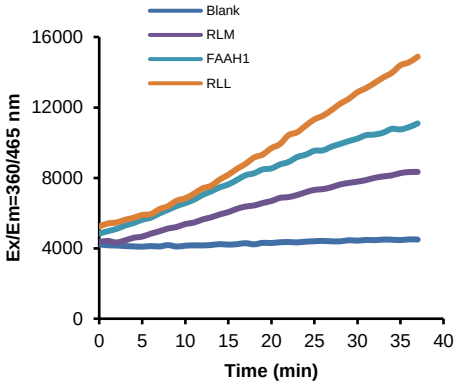


**Figure 2**. Kinetic measurement of FAAH activity. Lysates prepared from blank (no sample), rat liver microsome (RLM: 32 µg) and rat liver lysate (RLL: 80 µg) and FAAH Positive Control.

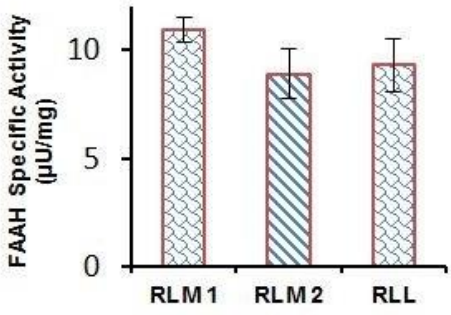


**Figure 3**. FAAH specific activity. Activity of two rat liver microsome preparations (RLM1 and RLM2) and rat liver lysate (RLL: 80 µg).

## 11. Notes

## Technical Support



**For all technical or commercial enquiries please go to:**
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