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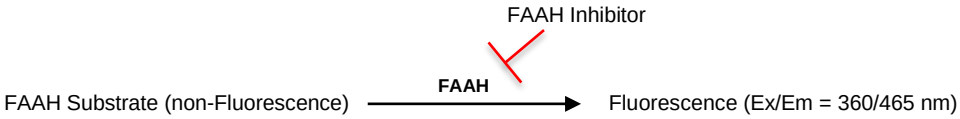
FAAH1 Inhibitor Screening Kit (Fluorometric)

rev 02/21

(Catalog # K379-100; 100 assays; Store at -20 °C)

I. Introduction:

Fatty Acid Amide Hydrolase (FAAH; EC: 3.5.1.99), also known as Oleamide Hydrolase or Anandamide Amidohydrolase is a member of the serine hydrolase family of enzymes. It is an integral membrane enzyme that hydrolyzes the endocannabinoid anandamide and related amidated signaling lipids. Endocannabinoids are endogenous lipid ligands that activate the Cannabinoid G-protein coupled Receptors (CB). CB1 and CB2 modulate physiological and behavioral processes such as pain, anti-inflammation etc. In addition to hydrolyzing Endocannabinoids, FAAH regulates other lipid signaling molecules with anti-inflammatory and analgesic properties. Recent study suggests that FAAH may represent an attractive therapeutic target for treatment of pain, inflammation etc. In **BioVision's Human FAAH1 Inhibitor Screening Kit,** FAAH1 hydrolyzes a non-fluorescent substrate to generate a fluorescent product, 7-amino-4-methylcoumarin, which can be measured at Ex/Em = 360/465 nm. This reaction is impeded in the presence of FAAH1 Inhibitor. The assay is high-throughput adaptable and can be completed in less than 1 hr.



II. Application:

- Screening/characterizing/studying potential inhibitors of Human FAAH1

III. Kit Contents:

Components	K379-100	Cap Code	Part Number
FAAH1 Assay Buffer	25 ml	WM	K379-100-1
FAAH1 Substrate	100 µl	Amber	K379-100-2
Human FAAH1	2 vials	Red	K379-100-3
FAAH1 Inhibitor (JZL 195)	100 µl	Blue	K379-100-4

IV. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer
- Multi-channel pipette

V. Storage Conditions and Reagent Preparation:

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

- **FAAH1 Assay Buffer:** Allow the Assay Buffer to warm to RT before use.
- **FAAH1 Substrate (in DMSO) and FAAH1 Inhibitor (in DMSO):** Store at -20 °C. Avoid repeated freeze/thaw cycles. Thaw these vials completely at RT and mix well before use. Use within two months.
- **Human FAAH1:** Reconstitute each vial in 110 µl of Assay Buffer. Mix well and keep on ice while in use. Avoid repeated freeze/thaw cycles. Aliquot and store at -80 °C. Use within two months.

VI. Human FAAH1 Inhibitor Screening Protocol:

1. **Test Inhibitor Preparation:** Dissolve the Test Inhibitor(s) in appropriate solvent at highest concentration to be tested. Dilute to 2X the desired test concentration with FAAH1 Assay Buffer. Add 50 µl diluted Test Inhibitor(s) into desired wells of a 96-well white plate designated as **Sample (S).**
   Note: Solvents used to solubilize the Test Inhibitors might affect the enzymatic activity. Prepare a **Solvent Control (SC)** well with the same final concentration of solvent used to dissolve the Test Inhibitor(s).
2. **Inhibitor Control, and Enzyme Control Preparation:** For **Enzyme Control (EC)** and **Background Control (BC),** add 50 µl FAAH1 Assay Buffer in each well. For Solvent Control, add 50µl of the final Solvent concentration in FAAH1 Assay Buffer. For FAAH1 **Inhibitor Control (IC)** well, dilute FAAH1 Inhibitor 100 times by adding 2 µl of FAAH1 Inhibitor to 198 µl FAAH1 Assay Buffer. Add 50 µl of the diluted FAAH1 Inhibitor into IC well(s).
3. **Human FAAH1:** Dilute the reconstituted FAAH1 to 4-fold by adding 20 µl of reconstituted FAAH1 to 60 µl of Assay Buffer. Then add 8 µl of the reconstituted FAAH1 into **S, EC, SC** and **IC** wells. Make enough Human FAAH1 solution for the number of assays to be performed. Add 8 µl FAAH Assay Buffer into BC wells. Mix well. Incubate at 25 °C for 5 min.
4. **FAAH1 Substrate Mix:** Dilute the substrate 50 times by adding 10 µl of substrate to 490 µl assay buffer. Make enough diluted substrate for the number of assays to be performed. Add 42 µl of diluted substrate to EC, SC, IC, BC & S wells respectively. Mix well.
5. **Measurement:** Shake for 30 sec**.** Measure fluorescence (Ex/Em = 360/465 nm) in a kinetic mode for 60 min at 37 °C.
6. **Calculations:** Choose any two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding fluorescence values (RFU_1 & RFU_2). Calculate the slope $\Delta RFU/\Delta T$ for all Samples including S, EC, by dividing the net $\Delta RFU = (RFU_2-RFU_1)$ value by the time Δt (t_2-t_1). If Solvent Control (SC) value is significantly different than that of EC replace the value of EC in the formulas below with the SC values.

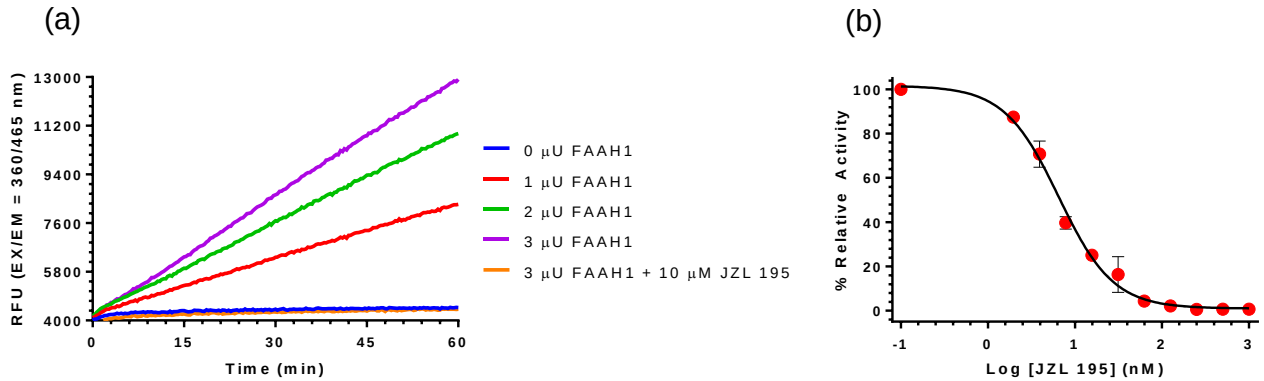
Calculate % relative inhibition as follows:

$$\textbf{Relative Activit} \quad (\%) = [(\textbf{Slope of S})/(\textbf{Slope of EC})]\ \textbf{X 100}$$

$$\textbf{Relative Inhibition } (\%) = [(\textbf{Slope of EC} - \textbf{Slope of S})/(\textbf{Slope of EC})]\textbf{X100}$$

Where: **Slope of EC is the slope of Enzyme Control**

Slope of S is the slope of Candidate Inhibitor



Figures: (a). FAAH1 activity assay in the presence and absence of FAAH1 inhibitor, JZL 195. (b). Dose-response curve for the inhibition of Human FAAH1 activity by JZL 195 (IC_{50} = 6.5 nM). Assay was performed following the kit protocol.

VII. Related Products:

Monoacylglycerol Lipase (MAGL) Activity Assay Kit (K561)
Cyclooxygenase (COX) Activity Assay Kit (Fluorometric) (K549)
FAAH Activity Assay Kit (K434)
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)

FOR RESEARCH USE ONLY! Not to be used on humans.