ab283359 – HDAC5 Inhibitor Screening Kit (Fluorometric)

For the screening of potential HDAC5 inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283381

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 6 months.

Materials Supplied

Item	Quantity	Storage Condition
FAAH1 Assay Buffer	25 mL	-20°C
FAAH1 Substrate	100 µl	-20°C
Human FAAH1	2 vials	-20°C
FAAH1 Inhibitor Control	100 µ	-20°C

Materials Required, Not Supplied

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

- Multi-well spectrophotometer
- 96-well clear plate with flat bottom
- Dounce Tissue Homogenize

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

FAAH1 Assay Buffer: Warm to room temperature (RT) before use.

FAAH1 Substrate (in DMSO) and FAAH1 Inhibitor (in DMSO): FAAH1 Substrate and inhibitor at room temperature and mix well. 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.

Assay Protocol

Test Inhibitor preparation:

1- Dissolve the Test Inhibitor(s) in appropriate solvent at highest concentration to be tested.
2- Dilute to 2X the desired test concentration with FAAH1 Assay Buffer.
3- 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).

Inhibitor Control, and Enzyme Control Preparation:

1- For Enzyme Control (EC) and Background Control (BC), add 50 µl FAAH1 Assay Buffer in each well.
2- For Solvent Control, add 50µl of the final Solvent concentration in FAAH1 Assay Buffer.
3- For FAAH1 Inhibitor Control (IC) well, dilute FAAH1 Inhibitor 100 times by adding 2 µl of FAAH1 Inhibitor to 198 µl FAAH1 Assay Buffer.
4- Add 50 µl of the diluted FAAH1 Inhibitor into IC well(s).

Human FAAH1

1- Dilute the reconstituted FAAH1 to 4-fold by adding 20 µl of reconstituted FAAH1 to 60 µl of Assay Buffer.
2- Add 8 µl of the reconstituted FAAH1 into S, EC, SC and IC wells.
3- Make enough Human FAAH1 solution for the number of assays to be performed.
4- Add 8 µl FAAH Assay Buffer into BC wells.
5- Mix well and incubate at 25 °C for 5 min.

FAAH1 Substrate Mix:

1- Dilute the substrate 50 times by adding 10 µl of substrate to 490 µl assay buffer.
2- Make enough diluted substrate for the number of assays to be performed.
3- Add 42 µl of diluted substrate to EC, SC, IC, BC & S wells respectively.
4- Mix well.

Measurement

Shake for 30 seconds. Measure fluorescence (Ex/Em = 360/465 nm) in a kinetic mode for 60 minutes at 37 °C.

Calculation:

1- Choose any two time points (t1 & t2) in the linear range of the plot and obtain the corresponding fluorescence values (RFU1 & RFU2).
2- Calculate the slope ΔRFU/ΔT for all Samples including S, EC, by dividing the net ΔRFU= (RFU2-RFU1) value by the time Δt (t2-t1).
3- 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.

$$\% \text{ Inhibition} = \frac{slope\ of\ enzyme\ control - slope\ of\ compound}{slope\ of\ (enzyme\ control)} \times 100$$

$$\% \text{ Relative activity} = \frac{slope\ of\ (test\ compound)}{slope\ of\ (enzyme\ control)} \times 100$$

Note: Slope of EC is the slope of Enzyme Control.
Slope of S is the slope of Candidate Inhibitor.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)