ab283385 – HDAC6 Inhibitor Screening Kit (Fluorometric)

For the screening of potential HDAC6 inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283385

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Storage and Stability

On receipt entire assay kit should be stored at -80°C protected from light. Upon opening storage conditions change as described below. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer XXXIX/HDAC6 Assay Buffer	25 mL	-20°C
Human HDAC6	10 µL	-80°C
HDAC Substrate III/HDAC6 Substrate	0.2 mL	-20°C
Developer II/Developer	1 mL	-20°C
HDAC6 Inhibitor/HDAC6 Inhibitor (Tubacin, 1mM)	20 µL	-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 spectrofluorometer
- 96-well white plate

Reagent Preparation

- Before using the kit, briefly centrifuge small vials at low speed prior to opening.

Assay Buffer XXXIX/HDAC6 Assay Buffer: Bring to room temperature (RT) before use. Store at 4 °C or -20°C.

HDAC Substrate III/HDAC6 Substrate and HDAC6 Inhibitor/Inhibitor: Store at -20°C

Developer II/HDAC6 Developer: Store at -20°C. Thaw on ice before use.

Human HDAC6: Aliquot and store at -80°C, avoid repeated freeze thaw cycles. Thaw on ice before use. Unused enzyme must be stored at -80°C immediately.

Assay Protocol

HDAC6 Enzyme Working Solution Preparation:

1. Prepare HDAC6 Enzyme Working Solution fresh by diluting Human HDAC6 enzyme 500X in the Assay Buffer XXXIX/HDAC6 Assay Buffer (e.g. 1 µL in 0.5 mL, enough for 10 samples). Based on number of samples, calculate appropriate amount of HDAC6 needed for dilution.
2. For each well Enzyme Control (EC), Sample (S), Inhibitor Control (IC), use 50 µL of the diluted HDAC6 Enzyme Working Solution in a microplate well. For Background Control (BC) use 50 µL Assay Buffer XXXIX/Assay Buffer in a microplate well.

Compounds, Inhibitor Control & Enzyme Control Preparations:

1. Dissolve candidate inhibitors at 50X highest final test concentration using preferred solvent.

2. Add 2 µL of test inhibitors (S) or Assay Buffer XXXIX/HDAC6 Assay Buffer (EC or BC) or 2 µL HDAC6 Inhibitor/HDAC Inhibitor (IC) into HDAC6 enzyme containing well(s). Cover the plate and incubate at 37°C for 15 min.

Δ Note: *Many commonly used organic solvents can severely impact enzymatic activity; e.g. DMSO may cause significant inhibition of HDAC6 activity at final concentrations of >5% (v/v). We recommend testing a parallel Solvent Control (SC) well with the same final concentration of solvent used to solubilize test ligands containing the same amount of enzyme as EC.*

Substrate Mix:

1. Prepare 48 µL of HDAC6 Substrate Mix containing:

	Substrate Mix
Assay Buffer XXXIX/HDAC6 Assay Buffer	46 µL
HDAC Substrate III/HDAC6 Substrate	2 µL

2. Dissolve the Substrate Mix by vigorous vortexing.
3. Add 48 µL of the Substrate Mix to wells containing the background control (BC), enzyme control (EC), inhibitor control (IC), solvent control (SC), and Samples (S). Mix well, cover the plate and incubate at 37°C for 30 min. To stop the reaction, add 10 µl of Developer II/Developer to each well. Incubate at 37°C for 10 min to generate fluorescence. After generation the fluorescence signal is stable for at least 20 min.

Measurement

Measure the fluorescence at Ex/Em= 380/490 nm in an endpoint mode at 37°C.

Calculation:

1. Obtain the fluorescence values for the BC, EC, S, SC, and IC. Subtract the fluorescence of BC from all Samples (S) including EC and IC.
2. Calculate % inhibition by Samples (S) and Inhibitor Control (IC) as given below. Use SC values instead of EC in case they are significantly different

$$\% \text{ Relative activity} = \frac{RFU\ of\ (S)}{RFU\ of\ (EC)} \times 100$$

$$\% \text{ Relative Inhibition} = \frac{RFU\ of\ (EC) - RFU\ of\ (S)}{RFU\ of\ (EC)} \times 100$$

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)