ab283380 – HDAC8 Inhibitor Screening Kit (Fluorometric)

For the screening of potential HDAC8 inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283380

Storage and Stability

On receipt store the vial of HDAC8 Enzyme at -80°C, and the rest of the kit should be stored at -20°C, protected from light. Upon opening, store the HDAC8 Assay Buffer at 4°C and use kit within 2 months.

Materials Supplied

Item	Quantity	Storage Condition
HDAC8 Assay Buffer	25 mL	-20°C
HDAC8 Substrate	200 µl	-20°C
HDAC8 Developer	1 mL	-20°C
HDAC8 Enzyme	200 µl	-80°C
HDAC8 Inhibitor (Trichostatin A)	200 µl	-20°C

Reagent Preparation

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

HDAC8 Assay Buffer: Warm to room temperature (RT) before use.

HDAC Substrate: Upon thawing aliquot and store at -20°C

Assay Protocol

Enzyme preparation:

1. For each well, prepare a total 50 µL HDAC8 Enzyme Solution:

	Enzyme Solution
HDAC8 Assay Buffer	48 µL
HDAC8 Enzyme	2 µL

Screen compounds, inhibitor control and blank control preparations:

1. Dissolve candidate inhibitors into a proper solvent.
2. Dilute to 4X the desired test concentration with HDAC8 Assay Buffer.
3. For Trichostatin A Inhibitor Control, dilute 1/9 with HDAC8 Assay Buffer.
4. Add 25 µL of diluted test inhibitors, Trichostatin A or HDAC8 Assay Buffer into HDAC8 enzyme wells as sample screen, Inhibtor Control (Trichostatin A), or Enzyme Control (No Inhibitor).
5. Mix well, and incubate for 10 minutes at 37°C.

Background well:

1. Add 75 µL HDAC8 Assay Buffer into a blank well.

Substrate Preparation:

1. For each well, prepare a total 25 µL Substrate Solution:

	Substrate Mix
HDAC8 Assay Buffer	23 µL
HDAC8 Substrate	2 µL

2. Mix, and add 25 µL of the substrate to all sample wells.
3. Mix, and incubate for 60 minutes at 37°C.

Developer:

1. Add 10 µL into each well (including background well); Mix well.
2. Incubate for 5 minutes at 37°C.

Measurement

Read Ex/Em=380/500 nm as R_{BG}, R_S, and R_{EC} for background well, sample wells or inhibitor control and enzyme control wells, respectively.

Calculation:

The RFU of fluorescence generated by hydrolyzation of substrate is ΔRFU of samples (or inhibitor control) ΔRFU S = $R_S - R_{BG}$, ΔRFU of Enzyme Control = ΔRFU EC = $R_{EC} - R_{BG}$. Set the ΔRFU EC as the 100%, and calculate the relative % Inhibition of the test inhibitors as:

$$\% \text{ Inhibition} = \frac{\Delta RFU\ EC - \Delta RFU\ S}{\Delta RFU\_EC} \times 100\%$$

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)