ab283378 – HDAC Inhibitor Drug Screening Kit (Fluorometric)

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

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283378

Storage and Stability

On receipt entire assay kit should be stored at -80°C, protected from light. Upon opening. Avoid repeated freeze/thaw for all non-buffer components.

Materials Supplied

Item	Quantity	Storage Condition
HDAC Substrate	500 µL	-80°C
10X HDAC Assay Buffer	1 mL	-80°C
Lysine Developer	1 mL	-80°C
HDAC Inhibitor (Trichostatin A, 1 mM)	10 µL	-80°C
HeLa Nuclear Extract (5 mg/ml)	200 µL	-80°C

Materials Required, Not Supplied

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

- 96-well plate with flat bottom. White plates are preferred for this assay.
- Fluorescence microplate reader.

Assay Protocol

Before using the kit, spin the tubes prior to opening. Warm HDAC Assay Buffer to room temperature (RT) before use.

Screen compounds, Inhibitor Control and Positive Control Preparations:
Dissolve candidate inhibitors into proper solvent.
Dilute to 2X the desired test concentration with ddH20.
Add 50 µl of diluted candidate inhibitor into well(s).
For Positive Control, add 50 µl ddH20 only.
For Negative Control, add 48 µl of ddH20 and 2 µl of Trichostatin A.

Reaction Mix Preparation:
Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

Item	Volume
10X HDAC Assay Buffer	10 µL
HeLa Nuclear Extract	2 µL
HDAC Substrate	5 µL
ddH20	33 µL

Mix well. Add 50 µl of the Reaction Mix into each well. Mix well. Incubate plate at 37°C for 30 min (or longer if desired).
Stop the reaction by adding 10 □l of Lysine Developer and mix well. Incubate the plate at 37°C for 30 min.

Measurement:

Read sample in a fluorescence plate reader with Ex. = 350-380 nm and Em. = 440-460 nm. Signal should be stable for several hours at RT.

Calculations:

Set the RFU of Positive Control as the 100%, and calculate the relative activity remains with candidate compounds as follow.

$$\text{Activity Remaining With Candidate Compounds} = \frac{RFU\ of\ candidate}{RFU\ of\ Positive\ Control} \times 100$$

Technical Support

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