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

All components in this kit are shipped on blue ice and are suitable for storage at -80°C, unless reconstituted. Upon receipt, immediately store kit at -80°C in the dark. Individual components may be stored at alternative temperatures as shown in the table below. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Upon opening. Avoid repeated freeze/thaw for all non-buffer components.

Materials Supplied

Item	Quantity	Storage Condition
HDAC Substrate	500 µL	-80°C
10X Assay Buffer 30	1 mL	-80°C or -20°C
Developer Mix M	1 mL	-80°C
HDAC Inhibitor	10 µL	-80°C
HeLa Nuclear Extract	200 µL	-80°C

PLEASE NOTE: 10X Assay Buffer 30 was previously labelled as 10X Assay Buffer XXX and 10X HDAC Assay Buffer, and Developer Mix M as Developer II and Lysine Developer, and HDAC Inhibitor as HDAC Inhibitor (Trichostatin A, 1 mM). The composition has not changed.

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 10X Assay Buffer 30 to room temperature (RT) before use.

Screen compounds, Inhibitor Control and Positive Control Preparations:
Dissolve HDAC Inhibitor/candidate inhibitors into proper solvent.
Dilute to 2X the desired test concentration with ddH20.
Add 50 µl of diluted HDAC Inhibitor/candidate inhibitor into well(s).
For Positive and Background Controls, 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	
	Reaction mix	Background Reaction mix
10X Assay Buffer 30	10 µL	10 µL
HeLa Nuclear Extract	2 µL	-
HDAC Substrate	5 µL	5 µL
ddH20	33 µL	35 µL

Mix well. Add 50 µl of the Reaction Mix into each well (positive control, negative control, candidate inhibitor). Mix well.
Add 50 µl of the Background Reaction Mix into Background Reaction Control Wells. Mix well.
Incubate plate at 37°C for 30 min (or longer if desired).
Stop the reaction by adding 10 µl of Developer Mix M 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.

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

Technical Support

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