

## Product datasheet

# Histone Deacetylase (HDAC) Activity Assay Kit (Fluorometric) ab156064

★★★★★ 1 Abreviews 29 References 5 Images

### Overview

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<b>Product name</b>	Histone Deacetylase (HDAC) Activity Assay Kit (Fluorometric)
<b>Detection method</b>	Fluorescent
<b>Sample type</b>	Cell culture extracts, Tissue Extracts
<b>Assay type</b>	Enzyme activity
<b>Assay time</b>	1h 00m
<b>Species reactivity</b>	<b>Reacts with:</b> Mammals
<b>Product overview</b>	Histone Deacetylase (HDAC) Activity Assay Kit (Fluorometric) ab156064 detects HDAC activity in lysates.

The HDAC Activity Assay Kit is primarily designed for the evaluation of HDAC inhibitors using a crude HDAC fraction. Also, any cultured primary cell, cell line, or tissue homogenate can be assayed for HDAC activity with the kit if the appropriate dose of HDAC specific inhibitor e.g. Trichostatin A is used.

The HDAC activity assay protocol is based on an acetylated peptide which is conjugated to AMC. AMC is a fluorescent dye and its fluorescence is quenched when conjugated to the peptide. When the HDAC de-acetylates the peptide, it becomes susceptible to cleavage by an enzyme (the Developer component). This then releases free AMC, which can be measured using a fluorescence microplate reader (Ex/Em 355/460 nm).

The HDAC assay has been shown to detect the activity of the HDAC family, at least class I HDACs in Human or animal cell lysates or in column fractions. The assay shows good linearity of sample response. The assay may be used to follow the purification of HDACs or may be used to detect the presence of HDACs in cell lysates.

### Notes

Applications for this kit include:

1. Monitoring the purification of HDACs including HDAC1, 2, 3 and 8.
2. Screening inhibitors or activators of HDACs.
3. Detecting the effects of pharmacological agents on HDACs.

### Background information on HDACs

Histone Deacetylases (HDACs) are a class of enzymes responsible for the deacetylation of lysine residues on the N-terminal part of the core histones (H2A, H2B, H3 and H4), allowing the histones

to wrap the DNA more tightly.

HDAC proteins occur in four groups (class I, class IIA, class IIB, class III, class IV) based on function and DNA sequence similarity.

Classes I, IIA and IIB are considered "classical" HDACs whose activities are inhibited by trichostatin A (TSA), whereas class III is a family of NAD<sup>+</sup>-dependent proteins (sirtuins) not affected by TSA. Class IV is considered an atypical class on its own, based solely on DNA sequence similarity to the others.

**Platform** Microplate reader

## Properties

**Storage instructions** Please refer to protocols.

Components	100 tests
Crude HDAC	1 x 500µl
Developer	1 x 500µl
Fluoro-Deacetylated Peptide (0.2 mM)	1 x 100µl
Fluoro-Substrate Peptide (0.2 mM)	1 x 500µl
HDAC Assay Buffer	2 x 1ml
HDAC Stop Solution	2 x 1ml
Trichostatin A (10µM)	1 x 500µl

**Function** Responsible for the deacetylation of lysine residues on the N-terminal part of the core histones (H2A, H2B, H3 and H4). Histone deacetylation gives a tag for epigenetic repression and plays an important role in transcriptional regulation, cell cycle progression and developmental events. Histone deacetylases act via the formation of large multiprotein complexes. Deacetylates SP proteins, SP1 and SP3, and regulates their function. Component of the BRG1-RB1-HDAC1 complex, which negatively regulates the CREST-mediated transcription in resting neurons. Upon calcium stimulation, HDAC1 is released from the complex and CREBBP is recruited, which facilitates transcriptional activation. Deacetylates TSHZ3 and regulates its transcriptional repressor activity. Deacetylates 'Lys-310' in RELA and thereby inhibits the transcriptional activity of NF-kappa-B.

**Tissue specificity** Ubiquitous, with higher levels in heart, pancreas and testis, and lower levels in kidney and brain.

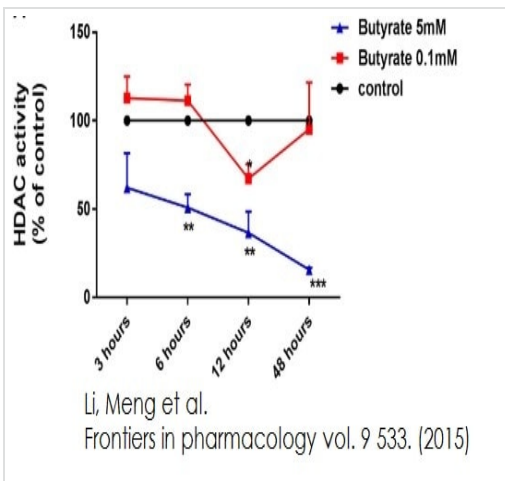
**Sequence similarities** Belongs to the histone deacetylase family. HD type 1 subfamily.

**Post-translational modifications** Sumoylated on Lys-444 and Lys-476; which promotes enzymatic activity. Desumoylated by SENP1.

Phosphorylation on Ser-421 and Ser-423 promotes enzymatic activity and interactions with NuRD and SIN3 complexes.

Ubiquitinated by CHFR, leading to its degradation by the proteasome.

**Cellular localization** Nucleus.

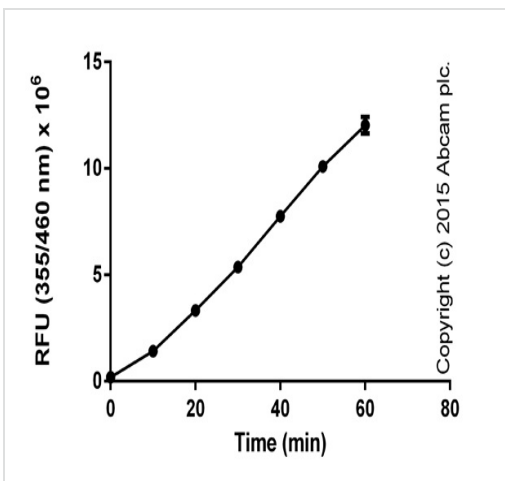


Functional Studies - Histone Deacetylase (HDAC)

Activity Assay Kit (Fluorometric) (ab156064)

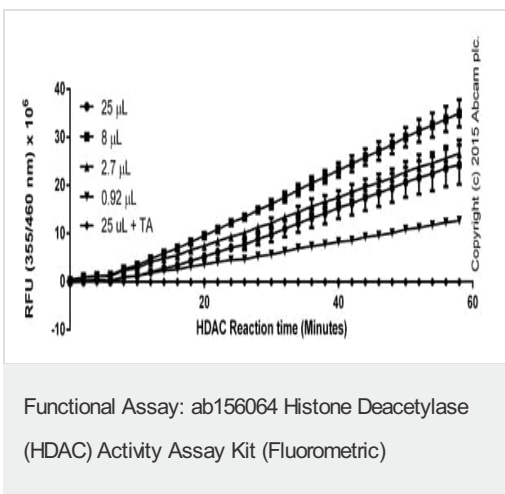
Li, Meng et al., Frontiers in pharmacology vol. 9 533., Fig 4, doi:10.3389/fphar.2018.00533

Butyrate, propionate, and TSA inhibited HDACs activity in HUVEC. HDACs activity were inhibited in HUVEC by treatment of butyrate and propionate. The results were normalized using the control as 100%.



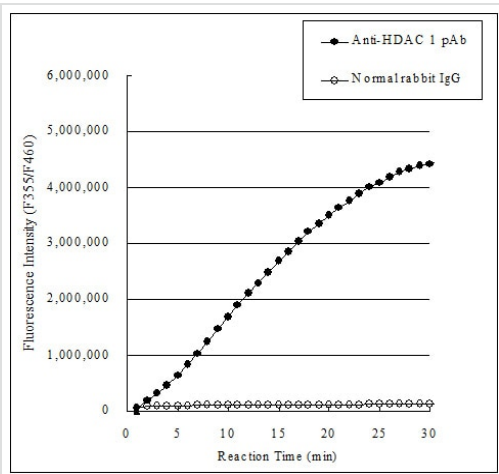
Functional Assay: ab156064 Histone Deacetylase (HDAC) Activity Assay Kit (Fluorometric)

8 uL (corresponding to 6.7e6 cells) of nuclear extracts (ab113474) from HL60 cells were assessed kinetically (+/- SD).



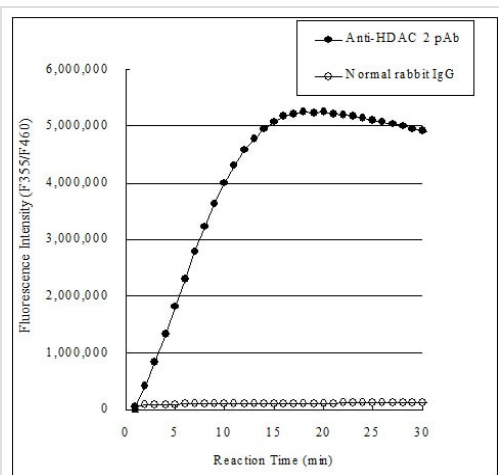
Functional Assay: ab156064 Histone Deacetylase (HDAC) Activity Assay Kit (Fluorometric)

Different volumes of positive control, with inhibitor (TA), kinetically read (+/- SD).



ab156064 - Histone Deacetylase (HDAC) Activity Assay Kit (Fluorometric)

Measurement of HeLa cell endogenous HDAC1 in an immunoprecipitated sample with anti-HDAC1 antibody and following the Histone Deacetylase (HDAC) Activity Assay Kit (Fluorometric) (ab156064) protocol.



ab156064 - Histone Deacetylase (HDAC) Activity Assay Kit (Fluorometric)

Measurement of HeLa cell endogenous HDAC2 in an immunoprecipitated sample with anti-HDAC2 antibody following the Histone Deacetylase (HDAC) Activity Assay Kit (Fluorometric) (ab156064) protocol.

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