# HDAC Fluorometric Assay Kits

**(Catalog# 107011, 100 assay)**

**Description:**

Histones are the chief protein components of chromatin. Modification of histone proteins through acetylation and deacetylation affects chromatin structure and regulates gene expression. Histone hyperacetylation is well correlated with increased transcription, whereas hypoacetylation correlates with transcriptional repression. Histone deacetylases (HDACs), catalyze the removal of acetyl groups from an N-acetyl lysine amino acid on a histone, generally resulting in transcriptional silencing. Histone deacetylases have been grouped into four classes. Class I (HDAC 1, 2, 3, 8) and Class II (HDAC 4, 5, 6, 7, and 9) are zinc-containing hydrolase's enzymes. The third class of HDACs consists of the members of the sirtuin family of enzymes (Sir 1 to 7). Class IV includes only HDAC11.

The HDAC Activity Assay Kit provides a convenient,homogeneous procedure for measuring HDAC activity from various sources of enzyme. It provides quantification of HDAC activity using a fluorogenic substrate. In HDAC assay, an acetylated substrate is incubated with HDAC-containing samples.Deacetylation of substrate sensitizes it to the HDAC developer, generates the AMC (7-amino-4-methylcoumarin) fluorophore, which can be detected at Ex354nm/Em442nm. The substrate included in the kit is cell-permeable, and the assay can measure HDAC activity directly in cell culture in a 96-well plate without a time-consuming cell extraction step. The kit also can be used for HTS of HDAC inhibitors with extracts or purified enzymes.

**Kit Contents for 100 Assays:**

1. Assay buffer 12 mL
2. Developer 12 mL
3. Substrate 100 µL
4. HDAC inhibitor (TSA) 10 µL 1mM

**Storage:**

Store the kit at –20°C protected from light. Shelf life: 12 months.

**PROCEDURES**

**A: Measuring HDAC activity using culture cell**

1. Culture $6x10^4$ cells in black 96-well tissue culture plate.

2. Prepare enough working solution by mix 40 µL assay buffer with 1µL substrate, and 10µL test compound for each well; Set up triplicate wells without compound to serve as the **positive control**, without substrate as the **blank control.**

3. Replace media with 50 µL working solution, and continue culture in a 30-37°C incubator for the desired time (0.5-2hr).
   Note: Set up triplicate wells with untreated cells to serve as a vehicle control. Add the same solvent used to deliver the test compounds to the vehicle control wells.
4. Stop the deacetylation reaction by adds 50µL/well of developer solution and mix thoroughly.
5. Incubate the plate an additional 15 min.
6. Measure fluorescent intensity for each well on a fluorescence plate reader. Options for fluorescence filter sets include 350–360nm for excitation and 440–450nm for fluorescence emission(Ex354nm/Em442nm).

**B: Screening HDAC inhibitor using nuclear extract.**

1. Prepare enough nuclear extract diluents: Using assays buffer dilute nuclear extract to 0.1-0.5 µg protein/µL, 40 µL is needed for per-assay.
2. Prepare enough working solution by mix 40 µL nuclear extract diluents with 1µL substrate for per-assay.
3. Prepare enough three-fold series dilute test compound with assay buffer, set up triplicate for each concentration, needs 10µL test compound for each assay.
4. Transfer 40uL working solution (triplicate) into 96-well black plate, add 10 µL series diluted test compound into plate. Set up triplicate wells without compound to serve as the positive control, without substrate as the blank control.
5. Incubate plate in a 30-37°C incubator for the desired time(0.5-2 hr).
6. Stop the deacetylation reaction by adds 50 µL/well of developer solution and mix thoroughly.
7. Incubate the plate an additional 15 min.
8. Measure fluorescent intensity for each well on a fluorescence plate reader (Ex 354 nm/Em 442 nm).

## CACULATION OF RESULTS

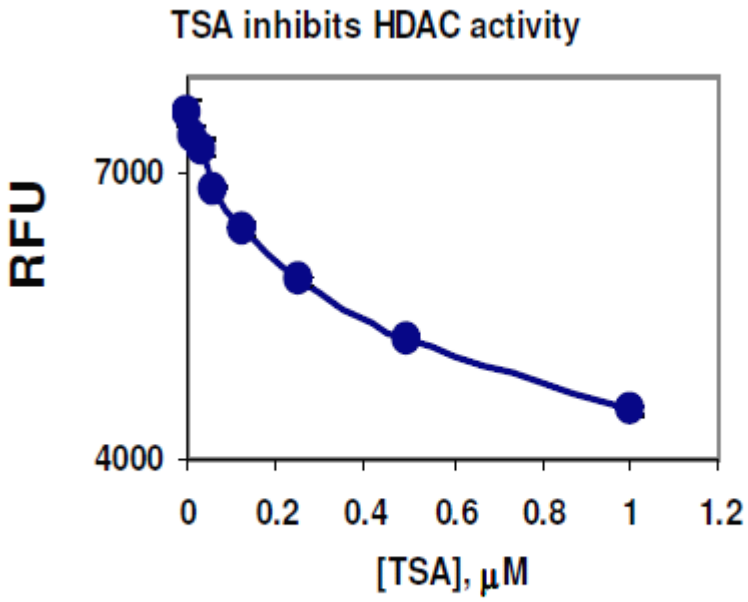
**Fluorescence Data** Subtract the average of fluorescence values of the blank control from all fluorescence values of experimental wells. Plot fluorescence vs. concentration of test compound.

Activity (%) = 100 x ($F_{sample}$ - $F_o$) / ($F_{ctrl}$ - $F_o$)

Where $F_{sample}$ and $F_{ctrl}$ are the average fluorescence intensities in the presence and absence (vehicle control) of the test compound and $F_o$ the averaged blank control fluorescence intensity.

**Materials needs but not supplied:**

Nuclear extract; 96-well black flat plate; Fluorescence plate reader.




Trichostatin A inhibition of HDACs activity in HeLa nuclear extract

**Note: This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals. Avoid contact with eye,skin and clothing. Do not ingest. Wear gloves.**

**RELATED PRODUCTS:**
Cell Viability Assay Kit (Cat #110001)
Protein Assay Kits (Cat #107001)
HDAC Colorimetric Assay Kit(Cat #107012)