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HDAC-5 Inhibitor Screening Kit (Fluorometric)

08/18

(Catalog # K171-100; 100 assays; Store at - 80ºC)

I. Introduction:

Histone Deacetylase 5 or HDAC-5 belongs to Class II Histone deacetylase family. It represses transcription by deacetylating lysine residues on the *N*-terminal part of histones H2A, H2B, H3 and H4. This epigenetic repression caused by HDAC-5, plays a role in transcriptional regulation, cell cycle progression and development. It interacts with myocyte enhancing factor (MEF-2) proteins that are associated with colon cancer, causing the repression of MEF-2 dependent gene expression. HDAC-5 is also involved in epigenetic regulation of ESR1 expression in breast cancer. HDAC-5 inhibition induces reactive oxygen species (ROS) production, ultimately leading to apoptotic cell death as well as mechanisms of mitochondrial quality control processes such as mitophagy and mitobiogenesis. Therefore, HDAC-5 inhibition in cancer cells significantly reduces tumor size due to apoptotic cell death of cancer cells (Hendrick, *et al*, Oncogene. 2017 Aug 24; 36(34):4859-4874), which makes HDAC-5 an attractive target for anti-cancer therapy. BioVision's HDAC-5 Inhibitor Screening Kit is a plate-based fluorometric kit for screening potential HDAC-5 inhibitors. The principle of the kit is based on the deacetylation of an acetylated substrate by HDAC-5. The deacetylated product is then cleaved by a developer to release a highly fluorescent moiety with Ex/Em= 380/460 nm. Trichostatin A, a well-known HDAC inhibitor is provided as inhibitor control.

Acetylated Substrate —HDAC-5→ Deacetylated Intermediate —HDAC-5 Developer→ Fluorescence (Ex/Em= 380/460 nm)

II. Applications:

- Screening of potential inhibitors of HDAC-5

III. Kit Contents:

Components	K171-100	Cap Code	Part Number
10X HDAC-5 Assay Buffer	1 ml	Green	K171-100-1
Enzyme Dilution Buffer	200 µl	Purple	K171-100-2
HDAC-5 Substrate	200 µl	Amber	K171-100-3
HDAC-5 Developer	1 ml	Orange	K171-100-4
HDAC-5 Enzyme	20 µl	Blue	K171-100-5
HDAC-5 Inhibitor (Trichostatin A)	20 µl	Yellow	K171-100-6

IV. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer
- Distilled Water
- Ice

V. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -80°C, protected from light. Briefly centrifuge small vials before opening. Read entire protocol before performing the assay. All components are stable for at least six months.

- **10X HDAC-5 Assay Buffer:** Warm to room temperature before use.
- **HDAC-5 Substrate, HDAC-5 Developer and HDAC-5 Inhibitor:** Thaw HDAC-5 substrate and inhibitor at **room temperature**, and HDAC Developer on ice before use. Aliquot and store at -20ºC in the dark. ***DO NOT EXPOSE HDAC-5 SUBSTRATE TO LIGHT.***
- **HDAC-5 Enzyme:** Thaw on ice before use. Aliquot and store at -80ºC, avoid repeated freeze thaw cycles. Prior to use, dilute 1 aliquot at a time at 1:10 in the provided enzyme dilution buffer **and keep on ice** at all times.

  Note: *Keep HDAC-5 Developer and HDAC-5 Enzyme on ice while performing the assay.*

VI. HDAC-5 Inhibitor Screening Assay Protocol:

NOTE: HDAC-5 is Temperature Sensitive. Set up assay(s) and plate on ice prior to Measurement (See Section VI; Step 4).

1. **Test Compound preparation:** Dissolve the test compound in appropriate solvent. Prepare at such concentration so volume of test compound solution added to a well is no more than 5 µl in the final 100 µl reaction volume per well. Add 2-5 µl test compound to each well of the 96-well white microplate. For "Solvent Control", add 2-5 µl of the solvent used to prepare test compound solution at its final concentration in test wells, and for "Inhibitor Control" add 2 µl of Trichostatin A, the provided HDAC-5 inhibitor. Bring up the volume to 50 µl in each well by adding distilled water. For the "Enzyme Control" add 50 µl distilled water to a well. Transfer the plate onto ice. **Note**: DMSO Concentration, up to 5% Final Concentration, does not adversely inhibit the activity of HDAC-5.
2. **Reaction Mix:** Mix enough reagents for the number of assays to be *performed, immediately before adding to the plate*. Prepare just enough for the number of reactions being rum immediately. Keeps the vial containing the reaction mix on ice while adding it to the well of the 96 well plate. For each well, prepare 30 µl Mix containing:

	Reaction Mix
10X HDAC-5 Buffer	5 µl
dH_2O	13 µl
HDAC Developer	10 µl
Diluted HDAC Enzyme	2 µl

Mix well and add 30 µl of the Reaction Mix to wells containing the Enzyme Control, Inhibitor Control, Solvent Control and Test Compounds. Incubate the plate on ice for no more than 5 minutes.

3. **Substrate Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 20 µl Substrate Mix containing:

	Substrate Mix
10X HDAC-5 Buffer	5 µl
dH_2O	13 µl
HDAC-5 Substrate	2 µl

Mix well and add 20 µl of the Substrate Mix to wells containing the Enzyme Control, Inhibitor Control, Solvent Control and Test Compounds while the plate is still on ice.

Note: Have the plate reader ready at Ex/Em= 380/460 nm on kinetic mode set to record fluorescence every 30 seconds at 37°C.

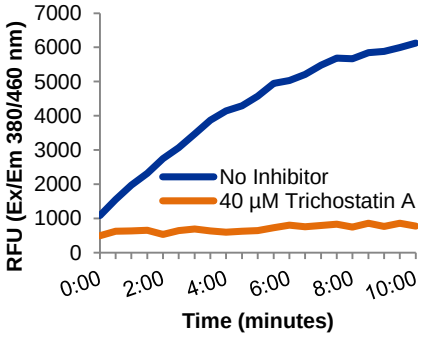
4. **Measurement:** Start recording fluorescence at Ex/Em= 380/460 nm after adding the substrate at 30 second intervals for 10-15 minutes at 37°C.

5. **Calculation:** Obtain Δ RFU for all test compounds by subtracting RFU obtained at two different time points within the linear range of the assay. Calculate slope for all samples, including "enzyme control" by dividing ΔRFU by time Δt ($t_2 - t_1$). If "Solvent Control" slope is different from "Enzyme Control" slope, use its values instead of "Enzyme Control" in the calculations shown below.

$$\% \text{ Inhibition} = \frac{[slope\ of\ (enzyme\ control) - slope\ of\ (test\ compound)]}{slope\ of\ (enzyme\ control)} X\ 100$$

$$\% \text{ Relative activity} = \frac{[slope\ (test\ compound)]}{slope\ (enzyme\ control)} X\ 100$$

a)



b)

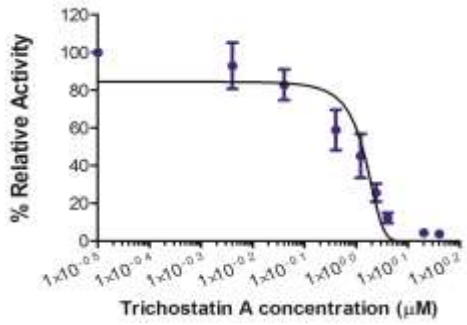


Figure: Inhibition of HDAC-5 enzyme activity by Trichostatin A. IC_{50} of Trichostatin A was determined to be 1.2 ± 0.2 µM. Assays were performed using kit protocol.

VII. RELATED PRODUCTS:

HDAC Activity Fluorometric Assay Kit K330
HDAC Activity Colorimetric Assay Kit K331
InSitu HDAC Activity Fluorometric Assay Kit K339
Human CellExp™ HDAC-5, Catalytic Domain, Human Recombinant, Active P1268
DiscoveryPak™ HDAC Inhibitor Set K851
DiscoveryPak™ HDAC Inhibitor Set II K876

FOR RESEARCH USE ONLY! Not to be used on humans.