



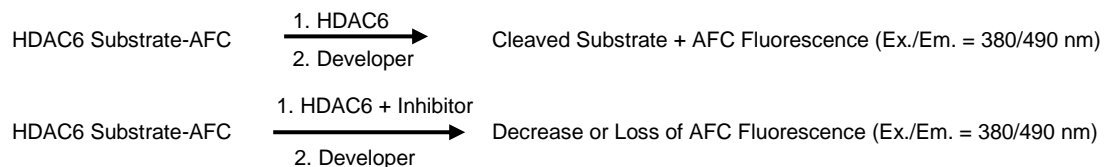
HDAC6 Inhibitor Screening Kit (Fluorometric)

07/17

(Catalog # K465-100; 100 assays, Store kit at -80°C)

I. Introduction:

Histone Deacetylase 6 (HDAC6, EC 3.5.1.98), is a class IIb HDAC enzyme that deacetylates an ϵ -N-acetyl lysine of histone and non-histone protein substrates. It is a unique member of HDAC family as it contains two deacetylase domains that are proposed to function independently of each other. HDAC6 can shuttle between the nucleus and cytoplasm, suggesting potential extra-nuclear functions by regulating the acetylation status of non-histone substrates. HDAC6 also affects transcription and translation by regulating the heat-shock protein 90 (Hsp90) and stress granules. Elevated HDAC6 activity is associated with cell motility and catalyzes increases α -tubulin deacetylation, thus influencing cancer cell metastasis. In addition, mutations in HDAC6 gene have been associated with Alzheimer's disease. HDAC6-selective inhibitors are considered as promising targets against autoimmune, inflammatory and oncology diseases such as breast and ovarian cancer. BioVision's HDAC6 Inhibitor Screening Kit utilizes deacetylase activity of HDAC6 towards a synthetic acetylated-peptide substrate resulting in the release of an AFC fluorophore, which can be easily quantified using a conventional microplate reader. In the presence of an HDAC6-selective inhibitor (Tubacin), the deacetylase activity of HDAC6 is reduced/abolished resulting in decrease or total loss of the fluorescence. This simple and high-throughput adaptable assay kit can be used to screen/study/characterize potential inhibitors of HDAC6.



II. Applications:

- Screening/characterizing inhibitors of HDAC6.

III. Kit Contents:

Components	K465-100	Cap Code	Part Number
HDAC6 Assay Buffer	25 ml	WM	K465-100-1
Human HDAC6	10 μ l	Green	K465-100-2
HDAC6 Substrate	0.2 ml	Red	K465-100-3
Developer	1 ml	Orange	K465-100-4
HDAC6 Inhibitor (Tubacin, 1 mM)	20 μ l	Blue	K465-100-5

IV. User Supplied Reagents and Equipment:

- 96-well white plate
- Multi-well spectrofluorometer

V. Storage Conditions and Reagent Preparation:

Store the entire kit at -80°C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

- **HDAC6 Assay Buffer:** Bring to room temperature before use. Store at 4°C or -20°C.
- **HDAC6 Substrate and Inhibitor:** Store at -20°C.
- **HDAC6 Developer:** Store at -20°C. Thaw on ice before use.
- **Human HDAC6:** Aliquot and store at -80°C. Avoid multiple freeze/thaw. Thaw on ice before use. Avoid repeated freeze/thaw, unused enzyme must be store at -80°C immediately.

VI. HDAC6 Inhibitor Screening Protocol:

1. HDAC6 Enzyme Working Solution Preparation: Prepare HDAC6 Enzyme Working Solution fresh by diluting Human HDAC6 enzyme 500X in the HDAC6 Assay Buffer (e.g. 1 μ l in 0.5 ml, enough for 10 samples). Based on number of samples, calculate appropriate amount of HDAC6 needed for dilution. For each well (Enzyme Control-**EC**, Sample-**S**, Inhibitor Control-**IC**), use 50 μ l of the diluted HDAC6 Enzyme Working Solution in a microplate well.

<u>EC, S and IC</u>	<u>Background Control (BC)</u>
50 μ l HDAC6 Enzyme Working solution	50 μ l HDAC6 Assay Buffer

2. Compounds, Inhibitor Control & Enzyme Control Preparations: Dissolve candidate inhibitors at 50X highest final test concentration using preferred solvent. Add 2 μ l of test inhibitors (**S**) or HDAC6 Assay Buffer (**EC** or **BC**) or 2 μ l HDAC Inhibitor (**IC**) into HDAC6 enzyme containing well(s). Cover the plate and incubate at 37°C for 15 min.

Note: Many commonly-used organic solvents can severely impact enzymatic activity; e.g. DMSO may cause significant inhibition of HDAC6 activity at final concentrations of >5% (v/v). We recommend testing a parallel Solvent Control (**SC**) well with the same final concentration of solvent used to solubilize test ligands containing the same amount of enzyme as EC.



3. HDAC6 Substrate Mix: Prepare 48 µl of HDAC6 Substrate Mix per well as given below:

- 46 µl HDAC6 Assay Buffer
- 2 µl HDAC6 Substrate

Dissolve the Substrate Mix by vigorous vortexing. Add 48 µl of HDAC6 Substrate Mix to Background Control (**BC**), Enzyme Control (**EC**), Inhibitor Control (**IC**) Solvent Control (**SC**) & Sample (**S**) wells. Mix well, cover the plate and incubate at 37°C for 30 min. To stop the reaction, add 10 µl of Developer to each well. Mix well, cover the plate and incubate at 37°C for 10 min to generate fluorescence. After generation, the fluorescence signal is stable for at least 20 min.

4. Measurement: Measure the fluorescence at Ex/Em 380/490 nm in an end point mode at 37 °C.

5. Calculations: Obtain the fluorescence values for the **BC**, **EC**, **S**, **SC**, and **IC**. Subtract the fluorescence of **BC** from all Samples (**S**) including **EC** and **IC**. Calculate % Inhibition by Samples (**S**) and Inhibitor Control (**IC**) as given below. Use **SC** values instead of **EC** in case they are significantly different

$$\% \text{ Relative Activity} = \frac{RFU \text{ of } S}{RFU \text{ of } EC} \times 100$$

$$\% \text{ Relative Inhibition} = \frac{RFU \text{ of } EC - RFU \text{ of } S}{RFU \text{ of } EC} \times 100$$

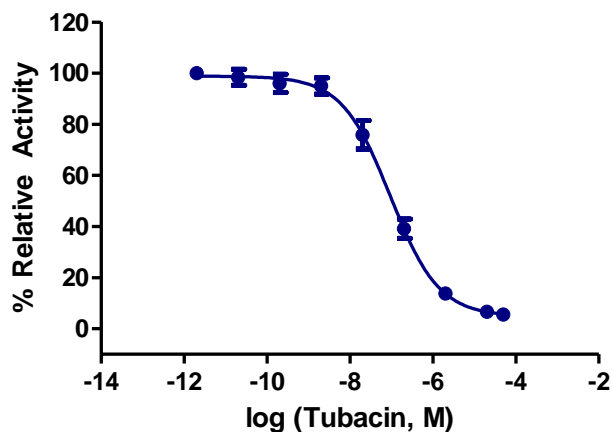


Figure: The inhibition of the enzymatic activity of HDAC6 in the presence of different concentrations of Tubacin, an HDAC6-selective inhibitor, is shown in the figure ($IC_{50} = 95.7 \text{ nM}$, $n = 4$). Data was fitted to a four parameter dose-response sigmoidal curve with variable slope using Graphpad Prism. The assays were performed according to the kit protocol.

VII. RELATED PRODUCTS:

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| HDAC Activity Colorimetric Assay Kit (K331) | HDAC Activity Fluorometric Assay Kit (K330) |
| HDAC Inhibitor Drug Screening Kit (Fluorometric) (K340) | HDAC1 Immunoprecipitation (IP) & Activity Assay Kit (K342) |
| HDAC2 Immunoprecipitation (IP) & Activity Assay Kit (K341) | HDAC3 Activity Fluorometric Assay Kit (K343) |
| HDAC3 Immunoprecipitation (IP) & Activity Assay Kit (K344) | HDAC3 Inhibitor Screening Kit (Fluorometric) (K363) |
| HDAC8 Activity Fluorometric Assay Kit (K348) | HDAC8 Inhibitor Screening Kit (Fluorometric) (K368) |

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