HDAC8 Inhibitor Screening Assay Kit (Fluorometric)

(Catalog #K368-100; 100 assays; Store kit at -80°C)

I. Introduction: Histone deacetylases (HDACs) represent a large family of enzymes identified as key regulators of nucleosomal histone acetylation, a major event that controls eukaryotic gene transcription and are classified into three groups. They are believed to be involved in important biological activities, such as cell differentiation, proliferation, apoptosis, and senescence. In BioVision's HDAC8 Inhibitor screening Kit, HDAC8 deacetylates the substrate (R-H-K(Ac)-K(Ac)-AFC) followed by an enzymatic cleavage to release the AFC molecule, which can be detected fluorometrically (Ex/Em = 380/500 nm). In the presence of an HDAC8 inhibitor, the deacetylation will be impeded which prevents the cleavage of the substrate to release AFC. The kit provides a rapid, simple, sensitive, and reliable test, which is also suitable for high throughput screening of HDAC8 inhibitors. Trichostatin A is included as a control inhibitor to compare the efficacy of the test inhibitors.

II. Kit Contents:

Components	100 assays	Cap Color	Part Number
HDAC-8 Assay Buffer	25 ml	WM	K368-100-1
HDAC-8 Substrate	200 µl	Red	K368-100-2
HDAC-8 Enzyme	200 µl	Green	K368-100-3
Developer	1 ml	Orange	K368-100-4
Trichostatin A (100 µM)	200 µl	Blue	K368-100-5

III. Storage and Handling:

Remove the vial of HDAC8 and store the kit at -20°C, protected from light. Allow HDAC8 Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

HDAC-8 Enzyme: Store enzyme at -80°C. Use within 2 months.

HDAC-8 Substrate: Upon thawing aliquot and store at -20°C, use within 2 months.

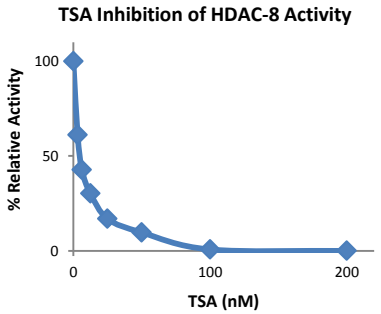
Developer and Trichostatin A: Store at -20°C; use within 2 months.

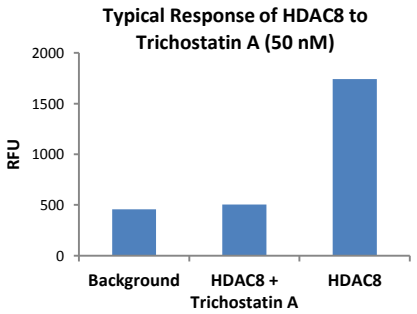
HDAC-8 Assay Buffer: Store at 4°C.

IV. HDAC-8 Inhibitor Screening Assay Protocol:

1. **Enzyme preparation:** For each well, prepare a total 50 µl HDAC-8 Enzyme Solution:
   48 µl HDAC8 Assay Buffer
   2 µl HDAC8 Enzyme
2. **Screen compounds, inhibitor control and blank control preparations:**
   Dissolve candidate inhibitors into a proper solvent. Dilute to 4X the desired test concentration with HDAC8 Assay Buffer. For Trichostain A Inhibitor Control, dilute 1:9 with HDAC8 Assay Buffer. Add 25 µl of diluted test inhibitors, Trichostatin A or HDAC8 Assay Buffer into HDAC8 enzyme wells as sample screen, Inhibitor Control (Trichostatin A), or Enzyme Control (No inhibitor). Mix well, and incubate for 10 minutes at 37°C.
3. **Background well:** Add 75 µl HDAC8 Assay Buffer into a blank well.
4. **Substrate preparation:** For each well, prepare a total 25 µl Substrate Solution:
   23 µl Assay Buffer
   2 µl Substrate
   Mix, add 25 µl of the substrate to all sample wells. Mix, incubate 60 min at 37°C.
5. **Developer:** Add 10 µl into each well (including background well); Mix well. Incubate for 5 min at 37°C.
6. **Measurement:** Read Ex/Em=380/500 nm as R_{BG}, R_S, and R_{EC} for background well, sample wells or inhibitor control and enzyme control wells, respectively.
7. **Calculation:** The RFU of fluorescence generated by hydrolyzation of substrate is ΔRFU of samples (or inhibitor control) ΔRFU S = $R_S - R_{BG}$, ΔRFU of Enzyme Control = ΔRFU EC = $R_{EC} - R_{BG}$. Set the ΔRFU EC as the 100%, and calculate the relative % Inhibition of the test inhibitors as:

$$\% \text{ Inhibition} = \frac{\Delta\text{RFU EC} - \Delta\text{RFU S}}{\Delta\text{RFU\_EC}} \times 100\%$$





RELATED PRODUCTS:

HDAC1 thru HDAC11 Antibodies
DiscoveryPak™ HDAC Inhibitor Set
HDAC Activity Assay Fluorometric Kit
HAT (P/CAF) Active Human Recombinant
Trichostatin A
HDAC8, human recombinant
HDAC3 Inhibitor Screening Assay Kit
HDAC Family Antibody Set
HDAC Activity Assay Colorimetric Kit
HAT Activity Assay Colorimetric Kit
HAT-1 thru HAT-3 Antibodies
HDAC8 Activity Assay Kit
HDAC3 Activity Assay Kit
HDAC3, human recombinant

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

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		