HDAC3 Inhibitor Screening Kit (Fluorometric)

(Catalog #K363-100; 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 HDAC3 Inhibitor screening Kit, HDAC3 and developer will deacetylate and cleave the substrate [Arg-His-Lys-Lys(Ac)-AFC] to release the AFC molecule, which can be detected by fluorometric method (Ex/Em = 380/500 nm). In presence of a HDAC3 inhibitor, the cleavage will be inhibited. The kit provides a rapid, simple, sensitive, and reliable test, which is also suitable for high throughput screening of HDAC3 inhibitors. Trichostatin A (TSA) is included as a control inhibitor to compare the efficacy of the test inhibitors.

II. Kit Contents:

Components	100 assays	Cap Color	Part Number
HDAC3 Assay Buffer	25 ml	WM	K363-100-1
HDAC3 Substrate	200 µl	Red	K363-100-2
HDAC3 Enzyme	200 µl	Green	K363-100-3
Developer	1 ml	Orange	K363-100-4
Trichostatin A	50 µl	Blue	K363-100-5

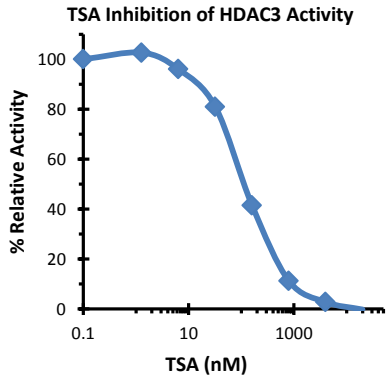
III. Storage and Handling:

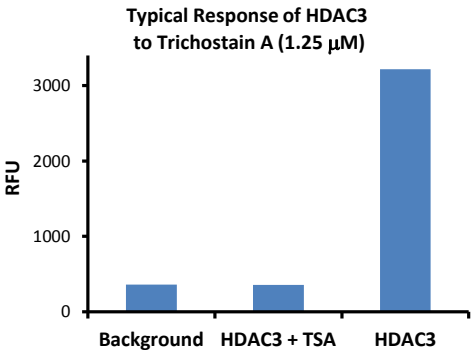
Remove the vial of HDAC3 and store the kit at -20°C, protect from light. **Store HDAC3 enzyme at -80°C and use within 2 months.** Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

IV. HDAC-3 Inhibitor Screening Assay Protocol:

1. **Enzyme solution:** Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl HDAC3 solution.
   48 µl Assay Buffer
   2 µl HDAC3 Enzyme
2. **Screen compounds, inhibitor control and blank control Preparations:**
   Dissolve candidate compounds into proper solvent. Dilute to 4X concentration by assay buffer. For Trichostatin A Inhibitor Control, dilute 1:9 with HDAC3 Assay Buffer. Add 25 µl of diluted test inhibitors, Trichostatin A Inhibitor Control or HDAC3 Assay Buffer into HDAC3 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 assay buffer into a blank well as background.
4. **Substrate solution 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 activity remains with test inhibitors as:

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





RELATED PRODUCTS:

HDAC1 thru HDAC-11 Antibodies
DiscoveryPak™ HDAC Inhibitor Set
HDAC Activity Assay Fluorometric Kit
HAT (P/CAF) Active Human Recombinant
Trichostatin A
HDAC8, human recombinant
HDAC8 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
	• 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.		