Product Information

HDAC Inhibitor Drug Screening Kit (Fluorometric)

I. Kit Contents:

Components	K2038-100 100 assays	Cap Color	Part Number
HDAC Substrate	500 µl	Amber	K2038-C-1
10X HDAC Assay Buffer	1.0 ml	Green	K2038-C-2
Lysine Developer	1.0 ml	Orange	K2038-C-3
HDAC Inhibitor (Trichostatin A, 1 mM)	10 ml	Blue	A8183
HeLa Nuclear Extract (5 mg/ml)	200 µl	Red	K2038-C-4

II. Introduction:

Histone deacetylase (HDAC) is an enzyme that removes acetyl group from a histone and plays an important role in regulating gene expression. Inhibition of HDAC can regulate transcription and induce apoptosis or differentiation in cancer cells. However, screening compounds that inhibit HDAC is difficult due to the lack of convenient way for detecting HDAC activity.

The HDAC Inhibitor Drug Screening Kit (Fluorometric) provides a fast and convenient way for screening of compounds for HDAC inhibition by detecting HDAC activity based on fluorescence method that eliminates radioactivity, chromatography or extractions in traditional assays. The assay needs only two easy steps performed on the same microtiter plate. First, the HDAC inhibitor candidates are mixed with HeLa Nuclear Extract and HDAC fluorometric substrate that contains an acetylated lysine side chain. Second, Deacetylation of the HDAC substrate sensitizes the substrate, so that further treatment with the Lysine Developer generates a fluorophore. The fluorophore can be easily detected using a fluorometer or a fluorescence plate reader. The assay is well suitable for high throughput screening applications.

III. Storage and Handling:

Store kit at -20℃, protected from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge all small vials before opening. Read the entire protocol before performing the assay. Aliquot & store HeLa nuclear extract at -70℃ to avoid loss of activity. The kit provides sufficient reagents for 100 positive control assays with the HeLa Nuclear Extract and 5 Negative Control assays with the HDAC Inhibitor, Trichostatin A.

IV. HDAC Assay Protocol:

1. Screen compounds, inhibitor control and positive control Preparations: Dissolve candidate inhibitors into proper solvent. Dilute to 2X the desired test concentration with ddH_2O. Add 50 µl of diluted candidate inhibitor into well(s). For positive control, add 50 µl ddH_2O only. For negative control, add 48 µl of ddH_2O and 2 µl of Trichostatin A.

2. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix
10X HDAC Assay Buffer	10 µl
HeLa Nuclear Extract	2 µl
HDAC Substrate	5 µl
ddH_2O	33 µl

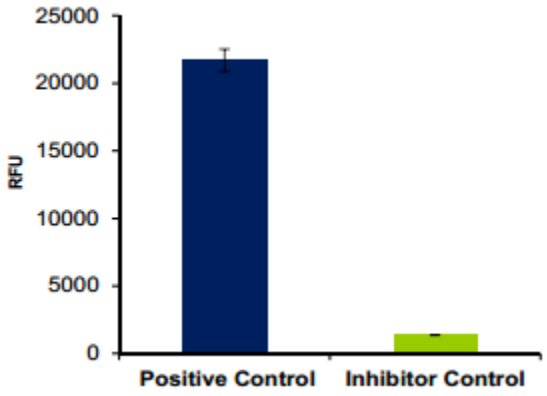
Mix well. Add 50 µl of the Reaction Mix into each well. Mix well. Incubate plate at 37℃ for 30 min (or longer if desired).

3. Stop the reaction by adding 10 µl of Lysine Developer and mix well. Incubate the plate at 37℃ for 30 min.

4. Measurement: Read sample in a fluorescence plate reader with Ex. = 350 - 380 nm and Em. = 440 - 460 nm. Signal should be stable for several hours at room temperature.

5. Calculation: Set the RFU of positive control as the 100%, and calculate the relative activity remains with candidate compounds as follow.

Activity remain with candidate compounds =RFU of Candidate/RFU of Positive Control X 100%.



General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the Nucleotide releasing buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures

		• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • 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.		

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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