# HDAC Inhibitor Drug Screening Kit (Fluorometric)

**(Catalog #K340-100; 100 assays; Store kit at -80°C)**

## I. Introduction:

Inhibition of histone deacetylase (HDAC) has been implicated to modulate transcription, to induce apoptosis or differentiation in cancer cells. However, screening of compounds for HDAC inhibition has been difficult due to the lack of convenient tools for analyzing HDAC activity. The new HDAC Inhibitor Drug Screening Kit provides a fast, fluorescence-based method that eliminates radioactivity, extractions, or chromatography, as used in traditional assays. The new procedure requires only two easy steps, both performed on the same microtiter plate. First, your inhibitor candidates are mixed with HeLa Nuclear Extract and HDAC fluorometric substrate, which comprises an acetylated lysine side chain. Deacetylation of the substrate sensitizes the substrate, so that, in the second step, treatment with the Lysine Developer produces a fluorophore. The fluorophore can be easily analyzed using a fluorescence plate reader or a fluorometer. The assay is well suited for high throughput screening applications.

## II. Kit Contents:

| Component | K340-100<br>100 assays | Color Code<br>Cap Color | Part<br>Number |
|---|---|---|---|
| HDAC Substrate | 500 µl | Amber | K340-100-1 |
| 10X HDAC Assay Buffer | 1.0 ml | Green | K340-100-2 |
| Lysine Developer | 1.0 ml | Orange | K340-100-3 |
| HDAC Inhibitor (Trichostatin A, 1 mM) | 10 µl | Blue | K340-100-4 |
| HeLa Nuclear Extract (5 mg/ml) | 200 µl | Red | K340-100-5 |

## III. Storage and Handling:

Store kit at -80°C, protected from light. Warm HDAC Assay Buffer to room temperature (RT) before use. Briefly centrifuge all small vials before opening. Read the entire protocol before performing the assay. Aliquot & store HeLa Nuclear Extract at -80°C 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:

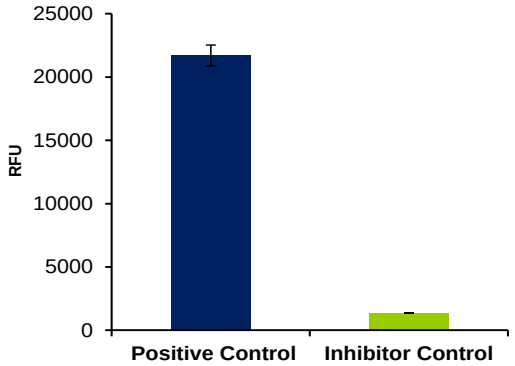
1. **Screen compounds, Inhibitor Control and Positive Control Preparations:** Dissolve candidate inhibitors into proper solvent. Dilute to 2X the desired test concentration with $ddH_20$. Add 50 µl of diluted candidate inhibitor into well(s). For Positive Control, add 50 µl $ddH_20$ only. For Negative Control, add 48 µl of $ddH_20$ and 2 µl of Trichostatin A.
2. **Reaction Mix Preparation:** 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 |
| $ddH_20$ | 33 µl |

Mix well. Add 50 µl of the Reaction Mix into each well. Mix well. Incubate plate at 37°C 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°C 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 RT.
5. **Calculation**: Set the RFU of Positive Control as the 100%, and calculate the relative activity remains with candidate compounds as follow.

$$\text{Activity remaining with candidate compounds} = \frac{\text{RFU of Candidate}}{\text{RFU of Positive Control}} \times 100\%$$




**RELATED PRODUCTS:**

- HDAC Fluorometric Assay kit
- HDAV Drug Screening Kit
- HDAC Inhibitors & Set
- HeLa Nuclear Extract
- HAT Activity Assay Kit
- Histone H2A, H2B, H3 & H4 Antibodies
- HDAC (1-11) Polyclonal Antibodies & Set

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***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 RT |
| | • 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 ; 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 (BV Cat# 1997) 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, deproteinize samples |
| | • Use of old or inappropriately stored samples | • Use fresh samples or store at correct temperatures till 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 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. | | |