Tribioscience

Histone deacetylase / HDAC Activity Fluorometric Assay (Catalog# TBS2050, 100 assay, Store at -20°C)

DESCRIPTION

Histones are the chief protein components of chromatin. They act as spools around which DNA winds. Covalent modification of histone proteins through acetylation and deacetylation affects chromatin structure and regulates gene expression. Histone hyperacetylation is well correlated with increased transcription, whereas hypoacetylation correlates with transcriptional repression. Histone deacetylases (HDACs) catalyze the removal of acetyl groups from an ε-N-acetyl lysine amino acid on a histone, and act as transcriptional repressors of genes. Histone deacetylases have been grouped into three classes. Class I (HDAC 1, 2, 3, 8) and Class II (HDAC 4, 5, 6, 7, and 9) are zinc-containing hydrolase enzymes. Class III consists of the members of the sirtuin family of enzymes (Sir 1 to 7). Inhibitors of HDAC classes I and II are being studied as a treatment for cancer and neurodegenerative diseases such as Huntington's and Alzheimer's diseases. The Sirtuin 1 (class III) enzyme represents a target for the treatment of age-related diseases and type II diabetes.

The Tribo™ HDAC Activity Assay Kit provides a fast, accurate, and simple approach for DAC activity detection from various sources of enzyme. The kit uses a fluorogenic substrate for HDAC activity detection. An acetylated substrate is incubated with HDAC-containing samples. Deacetylation of the substrate in the presence of the HDAC developer generates strong fluorescence, which can be detected at Ex354nm/Em442nm. The substrate included in the kit is cell-permeable, and the assay can measure HDAC activity directly in cell culture in a 96-well plate without a time-consuming cell extraction step. The kit also can be used for HTS of HDAC inhibitors with extracts or purified enzymes.

KIT CONTENTS (for 100 assay)

Assay buffer 12 mL; Developer 12 mL; Substrate 60 µL.

Storage conditions Store the substrate at –20°C protected from light. The other components may be stored at 4°C. Shelf life: 12 months.

PROCEDURES

A: Measuring HDAC activity using culture cell

1. Culture $6x10^4$ cells in black 96-well tissue culture plate.
2. Prepare enough working solution by mix 40 µL assay buffer with 1µL substrate, and 10µL test compound for each well; Set up triplicate wells without compound to serve as the **positive control**, without substrate as the **blank control**.
3. Replace media with 50 µL working solution, and continue culture in a 30°C incubator for the desired time (0.5-2hr).

Note: Set up triplicate wells with untreated cells to serve as a **vehicle control**. Add the same solvent used to deliver the test compounds to the vehicle control wells.

4. Stop the deacetylation reaction by adds 50µL/well of developer solution and mix thoroughly.
5. Incubate the plate an additional 15 min.
6. Measure fluorescent intensity for each well on a fluorescence plate reader at 350–360nm for excitation and 440–450nm for emission (Ex354nm/Em442nm).

B: Screening HDAC inhibitor using nuclear extract.

1. Prepare enough nuclear extract diluents: Using assay buffer dilute nuclear extract to 100 µg protein/mL, per-assay 40 µL is needed.
2. Prepare enough working solution by mix 40 µL nuclear extract diluents with 1µL substrate.
3. Prepare enough three-fold series dilute test compound with assay buffer, set up triplicate for each concentration, needs 10µL test compound for each assay.
4. Transfer 40uL working solution (triplicate) into 96-well black plate, add 10µL series diluted test compound into plate. Set up triplicate wells without compound to serve as the **positive control**, without substrate as the **blank control**.
5. Incubate plate in a 30°C incubator for the desired time (0.5-2hr).
6. Stop the deacetylation reaction by adds 50 µL/well of developer solution and mix thoroughly.
7. Incubate the plate an additional 15 min.
8. Measure fluorescent intensity for each well on a fluorescence plate reader (Ex354nm/Em442nm).

CACULATION OF RESULTS

Fluorescence Data Subtract the average of fluorescence values of the blank control from all fluorescence values of experimental wells. Plot fluorescence vs. concentration of test compound.

Activity (%) = 100 x (F_{sample} - F_o) / (F_{ctrl} - F_o)

Where F_{sample} and F_{ctrl} are the average fluorescence intensities in the presence and absence (vehicle control) of the test compound and F_o the averaged blank control fluorescence intensity.

RELATED PRODUCTS:

LDH Cytotoxicity Assay (TBS2002)
ATP Colorimetric/ Fluorometric Assay (TBS 2010)
ADP Colorimetric/Fluorometric Assay (TBS3020)
Cytochrome c Reductase Activity Assay (TBS2116)
CCK-8 Cell Viability Assay (TBS2022)
Cell Nuclear Extract kit (#TBS6025)
HDAC Activity Colorimetric Assay Kit (#TBS2065)
GOT Activity Assay (TBS2013)
Thiol Fluorometric Assay (TBS2026)
GSH Assay (TBS2028)
Homocysteine Fluorometric Assay (TBS2091)
NNMT Inhibitor Screening Assay (TBS2097)
G6PDH Activity Colorimetric Assay (TBS2102)
ATP Colorimetric/Fluorometric Assay Kit (TBS2010)
Caspase-3 Colorimetric Assay kit (TBS2030)
AOPI Viability Assay for Flow Cytometry (TBS2069)

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