Tribioscience

Histone Deacetylase / HDAC Colorimetric Assay Kits (Catalog: TBS2065, 100 assay, Store at -20°C)

DESCRIPTION

Histones are the chief protein components of chromatin, which 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, 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's enzymes. Class III of deacetylases consists of the members of the sirtuin family of enzymes (Sir 1 to 7). Inhibitors of HDAC classes II and I 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 treatment of age-related diseases and type II diabetes.

The Tribo™ HDAC Colorimetric Assay Kit provides a convenient, sensitive, and simple approach to quantify HDAC activity from various sources of enzyme using a colorimetric substrate. In HDAC assay, an acetylated substrate is incubated with HDAC-containing samples. Deacetylation of substrate sensitizes it in presence of HDAC developer, generates the colorimetric substrate, which can be detected at OD395nm. 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)

Component	Unit Size
Assay Buffer	12 mL
Substrate	1.2 mL
Developer	1.2 mL
Deacetylated Standard (10 mM)	20 µL
Positive Control	20 µL

Storage conditions Store the kit at –20°C.
Shelf life: 12 months.

PROCEDURES

A: Measuring HDAC activity

1. Prepare 50-200 µg of nuclear extract or cell lysate sample in final volume of 50 µL with Assay Buffer for each well. For negative control, add 50 µL Assay Buffer to the negative control well.
2. **(Optional)** For the positive control, take 5 µL of the HDAC Positive Control into 45 µL assay buffe in the positive control well.
3. Add 30 µL Assay Buffer, and 10 µL Substrate to each well, mix thoroughly.
4. Incubate at 37°C for 2 hours or longer.
5. Add 10 µL of developer solution to each well to stop the reaction, mix thoroughly.
6. Incubate at 37°C for 30 minutes.
7. Read plate in a plate reader at 395nm. HDAC activity can be expressed as the relative O.D. per µg protein sample. The calculation equation is below:

HDAC activity= (Sample OD – Negative OD)/Sample mass (µg)

B: Standard Curve Method

The Standard Curve Method using Deacetylated Standard is optional and should be performed as same as Procedure A.

1. Prepare standard concentrations as Table below. In the wells #1-7, add the HDAC Standard and assay buffer:

Well	1	2	3	4	5	6	7
Standard (µL)	0	0.5	1	2	3	4	5
Assay Buffer(µL)	10	9.5	9	8	7	6	5
nmoles /well	0	5	10	20	30	40	50

2. Add 80 µL Assay Buffer to the standard wells and mix thoroughly.
3. The procedures for test sample are same as the Procedure A.
4. Incubate at 37°C for 2 hours or longer.
5. Add 10 µL of developer solution to each well to stop the reaction, mix thoroughly.
6. Incubate at 37°C for 30 minutes.
7. Read the plate at 395nm, calculate the HDAC activity.
8. Calculate Δ OD 395 nm:

   Δ OD 395 nm =Standard OD – Standard zero OD).

9. Create the standard curve by plotting Δ OD 395 nm versus the deacetylated standard amount, and derive the resulting standard curve equation:

   Y= AX+B.

   Deacetylated amount (nmoles): X=(Y-B)/A

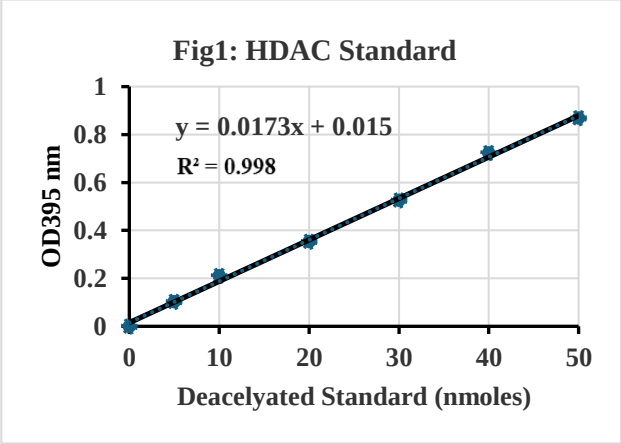
   Where Y is Δ OD 395 value, X is the deacetylated sample amount (nmole), A is the standard curve slope; B is a constant value.

   For Sample Δ OD 395 nm:
   Δ OD 395 nm. Sample = OD sample – OD negative).

10. HDAC Activity (pmole/min/ug) = (X * 1000)/ (T * S)

    Where T is incubation time (min), S: sample mass (µg).

11. Typical standard curve shown in Fig.1 as a reference. The Figure cannot be used for data analysis. It is just a reference.



RELATED PRODUCTS:

LDH Cytotoxicity Assay (TBS2002)
ATP Colorimetric/ Fluorometric Assay (TBS2010)
Caspase-1 Colorimetric Assay (TBS2040)
Caspase-3 Colorimetric Assay (TBS2030)
ADP Colorimetric/Fluorometric Assay (TBS3020)
Cytochrome c Reductase Activity Assay (TBS2116)
CCK-8 Cell Viability Assay (TBS2022)
Cell Nuclear Extract kit (TBS6025)
HDAC Fluorometric Assay Kit (TBS2060)
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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