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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
H87-31G-05	5 µg
H87-31G-10	10 µg

HDAC5, Active

Recombinant protein expressed in Sf9 cells

Catalog # H87-31G

Lot # W019-1

Product Description

Recombinant mouse HDAC5 (617-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is BC060609.

Gene Aliases

mHDA1; AI426555; mKIAA0600

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

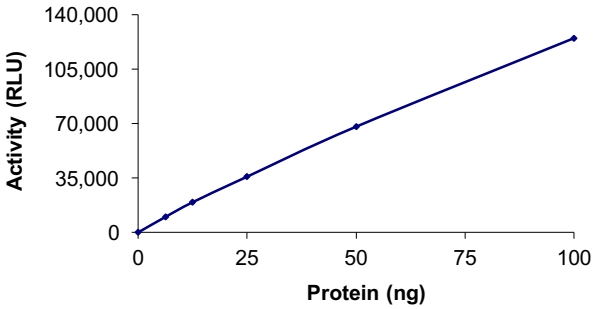
Scientific Background

HDAC5 or Histone deacetylase 5 belongs to the class II histone deacetylase/acuc/apha family that possesses histone deacetylase activity and represses transcription when tethered to a promoter (1). HDAC 5 plays a critical role in transcriptional regulation, cell cycle progression, and developmental events and also acts as a potential therapeutic target for the prevention of atherosclerosis. HDAC5 can co-immunoprecipitates with HDAC3 family members forming multicomplex proteins. HDAC5 can also interact with myocyte enhancer factor-2 (MEF2) proteins, resulting in repression of MEF2-dependent genes. HDAC5 gene is thought to be associated with colon cancer (2).

References

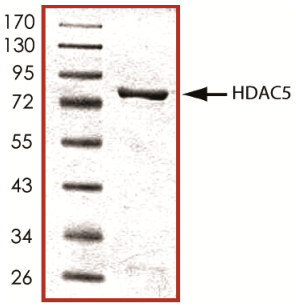
1. Grozinger, C. M. et.al : Three proteins define a class of human histone deacetylases related to yeast Hda1p. Proc. Nat. Acad. Sci. 96: 4868-4873, 1999.
2. Scanlan, M. J. et.al: Characterization of human colon cancer antigens recognized by autologous antibodies. Int. J. Cancer 76: 652-658, 1998.

Specific Activity



The specific activity of HDAC5 was determined to be **110 RLU/min/ng** as per activity assay protocol.

Purity



The purity of HDAC5 was determined to be **>90%** by densitometry.
Approx. MW **80kDa**.

HDAC5, Active

Recombinant protein expressed in Sf9 cells

Catalog Number	H87-31G
Specific Activity	110 RLU/min/ng
Specific Lot Number	W019-1
Purity	>90%
Concentration	0.1µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active HDAC5 (Catalog #: H87-31G)

Active HDAC5 (0.1μg/μl) diluted with HDAC-Glo I/II™ Buffer and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active HDAC5 for optimal results).

HDAC-Glo I/II™ Activity Assay Kit (Promega)

HDAC-Glo I/II™ Buffer, 25ml
HDAC-Glo I/II™ Substrate Cake, 1 bottle
HDAC-Glo I/II™ Developer Reagent, 10μl

Assay Protocol

The HDAC5 assay is performed using the HDAC-Glo I/II™ Activity Assay Kit (Promega), which is broadly used for assaying histone deacetylase class I and II enzymes. The Activity Assay Kit examines sequential reaction of deacetylation of an acetylated luminogenic peptide substrate by HDAC5, followed by the specific proteolytic cleavage of the deacetylated peptide by a developer enzyme and finally the firefly luciferase detection with the liberated aminoluciferin. The luminescent signal produced by the above steps is related to the activity of HDAC5.

Step 1. Thaw the Active HDAC5 and HDAC-Glo I/II™ Developer Reagent on ice.

Step 2. Thaw the HDAC-Glo I/II™ Buffer and HDAC-Glo I/II™ Substrate and equilibrate to room temperature.

Step 3. Prepare the following working solutions:

- Diluted active HDAC5 with HDAC-Glo I/II™ Buffer on ice
- Prepare the HDAC-Glo I/II™ Substrate Solution by adding 10ml of HDAC-Glo I/II™ Buffer to the HDAC-Glo I/II™ Substrate Cake bottle. (The aliquots can be refrozen if developer reagent has not been added).
- Prepare the HDAC-Glo I/II™ Reaction Reagent by adding 1μl of Developer Reagent to 1ml of Substrate Solution.

Step 4. In a polystyrene 96-well plate, add the following components to initiate the reaction:

Component 1. 20μl of diluted Active HDAC5 (Catalog #H87-31G)

Component 2. 20μl of HDAC-Glo I/II™ Reaction Reagent in step 3

Step 5. Set up a blank control as outlined in step 4 by excluding the addition of the diluted HDAC5 preparation. Replace the HDAC5 preparation with an equal volume of HDAC-Glo I/II™ Buffer.

Step 6. Incubate the mixture at room temperature for 15 minutes on a plate shaker.

Step 7. Read the polystyrene 96-well reaction plate using the KinaseGlo Luminescence Protocol on a GloMax plate reader (Promega; Cat# E7031).

Step 8. Determine the corrected activity (RLU) by removing the blank control value (see Step 5) for each sample and calculate the HDAC specific activity as outlined below.

HDAC Specific Activity (SA) (RLU/min/ng)

Corrected RLU from reaction / (Reaction time in min)*(Enzyme amount in ng)

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