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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
H92-31G-05	5 µg
H92-31G-10	10 µg

HDAC10, Active

Recombinant human protein expressed in Sf9 cells

Catalog # H92-31G

Lot # W175-2

Product Description

Recombinant human HDAC10 (1-482) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_032019.

Gene Aliases

DKFZp761B039; MGC149722

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.

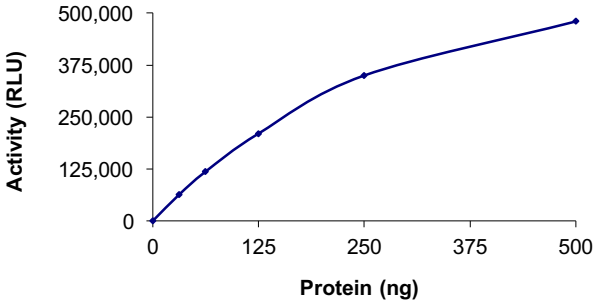
Scientific Background

HDAC10 belongs to the histone deacetylase/acuc/apha family and is a component of the histone deacetylase complex. HDAC10 belongs to class II of the histone deacetylase family that catalyzes the deacetylation of lysine residues in the N-terminal tail of histones and represses transcription in large multiprotein complexes with transcriptional co-repressors (1). Therefore, HDAC10 plays a role in transcriptional repression. HDAC10 interacts with HDAC3 in co-transfected embryonic kidney cells (2). Deletion analysis indicates that both the N- and C-terminal domains of HDAC10 bound HDAC3 independently.

References

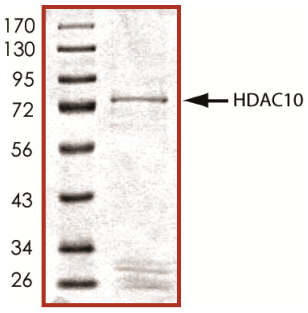
1. Fischer, D. D. et.al: Isolation and characterization of a novel class II histone deacetylase, HDAC10. J. Biol. Chem. 277: 6656-6666, 2002.
2. Tong, J. J. et.al: Identification of HDAC10, a novel class II human histone deacetylase containing a leucine-rich domain. Nucleic Acids Res. 30: 1114-1123, 2002.

Specific Activity



The specific activity of HDAC10 was determined to be **120 RLU/min/ng** as per activity assay protocol.

Purity



The purity of HDAC10 was determined to be **>80%** by densitometry.
Approx. MW **77kDa**.

HDAC10, Active

Recombinant human protein expressed in Sf9 cells

Catalog Number	H92-31G
Specific Activity	120 RLU/min/ng
Specific Lot Number	W175-2
Purity	>80%
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 HDAC10 (Catalog #: H92-31G)

Active HDAC10 (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 HDAC10 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 HDAC10 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 HDAC10, 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 HDAC10.

Step 1. Thaw the Active HDAC10 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 HDAC10 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 HDAC10 (Catalog #H92-31G)

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 HDAC10 preparation. Replace the HDAC10 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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