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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
H91-31G-05	5 µg
H91-31G-10	10 µg

HDAC9, Active

Recombinant human protein expressed in Sf9 cells

Catalog # H91-31G
Lot # W140-1

Product Description

Recombinant human HDAC9 (548-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_058176.

Gene Aliases

DKFZp779K1053; HD7; HDAC; HDAC7; HDAC7B; HDAC9B; HDAC9FL; HDRP; KIAA0744; MITR

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.

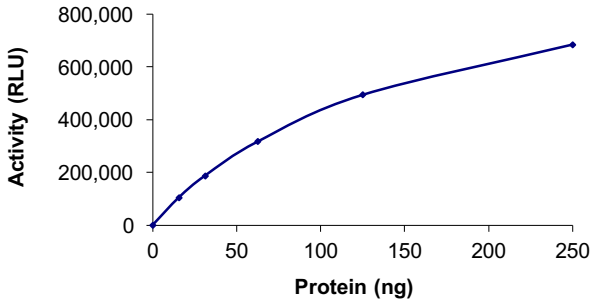
Scientific Background

HDAC9 or MIRT belongs to the histone deacetylase /acuc/apha family and is a component of the histone deacetylase complex. HDAC9 gene is orthologous to the Xenopus and mouse MITR genes (MEF2-interacting transcription repressor). HDAC9 represses MEF2 activity through recruitment of multicomponent corepressor complexes that include CtBP and HDACs. HDAC9 is a calcium-sensitive transcriptional repressor of MEF2 (1). Binding of calmodulin to HDAC9 leads to its dissociation from MEF2, relieving MEF2 from the transcriptional repression. HDAC9 is a signal-responsive transcriptional repressor in mouse skeletal muscle that is downregulated upon denervation (2).

References

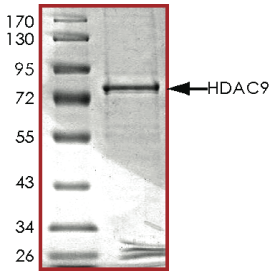
1. Youn, H-D. et al: Calcium regulates transcriptional repression of myocyte enhancer factor 2 by histone deacetylase 4. J. Biol. Chem. 275: 22563-22567, 2000.
2. Mejat, A. et al: Histone deacetylase 9 couples neuronal activity to muscle chromatin acetylation and gene expression. Nature Neurosci. 8: 313-321, 2005.

Specific Activity



The specific activity of HDAC9 was determined to be **260 RLU/min/ng** as per activity assay protocol.

Purity



The purity of HDAC9 was determined to be **>75%** by densitometry.
Approx. MW **77kDa**.

HDAC9, Active

Recombinant human protein expressed in Sf9 cells

Catalog Number	H91-31G
Specific Activity	260 RLU/min/ng
Specific Lot Number	W140-1
Purity	>75%
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.

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com
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FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.

Activity Assay Protocol

Reaction Components

Active HDAC9 (Catalog #: H91-31G)

Active HDAC9 (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 HDAC9 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 HDAC9 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 HDAC9, 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 HDAC9.

Step 1. Thaw the Active HDAC9 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 HDAC9 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 HDAC9 (Catalog #H91-31G)

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 HDAC9 preparation. Replace the HDAC9 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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