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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
H93-30G-05	5 µg
H93-30G-10	10 µg

HDAC11, Active

Recombinant full length protein expressed in Sf9 cells

Catalog # H93-30G

Lot # U1861-6

Product Description

Recombinant full length human HDAC11 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_024827.

Gene Aliases

FLJ22237; HD11

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.

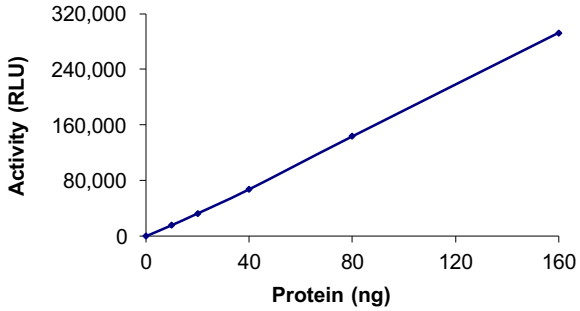
Scientific Background

HDAC11 belongs to the histone deacetylase/acuc/apha family and is a component of the histone deacetylase complex. HDAC11 belongs to class IV of the histone deacetylase family that localizes to the nucleus and is involved in regulating the expression of interleukin 10 (1). HDAC11 expression is the highest in brain, heart, skeletal muscle, kidney and testis. HDAC11 control the DNA expression by modifying the core histone octamers that package DNA into dense chromatin structures and repress gene expression. HDAC11 has HDAC activity and this activity is inhibitable by trapoxin, an HDAC inhibitor (2). In coimmunoprecipitation experiments, HDAC11 interacts with HDA6.

References

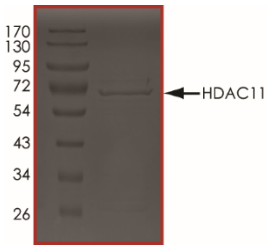
1. Villagra, A. et al: The histone deacetylase HDAC11 regulates the expression of interleukin 10 and immune tolerance. Nat Immunol. 2009 Jan;10(1):92-100.
2. Gao, L. et.al: Cloning and functional characterization of HDAC11, a novel member of the human histone deacetylase family. J. Biol. Chem. 277: 25748-25755, 2002.

Specific Activity



The specific activity of HDAC11 was determined to be **60 RLU/min/ng** as per activity assay protocol.

Purity



The purity of HDAC11 was determined to be **>85%** by densitometry.
Approx. MW **66kDa**.

HDAC11, Active

Recombinant full length protein expressed in Sf9 cells

Catalog #	H93-30G
Specific Activity	60 RLU/min/ng
Lot #	U1861-6
Purity	>85%
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 HDAC11 (Catalog #: H93-30G)

Active HDAC11 (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 HDAC11 for optimal results).

HDAC-Glo I/II™ Activity Assay Kit (Promega, Catalog #: G6420)

HDAC-Glo I/II™ Buffer (Catalog #: G648)
HDAC-Glo I/II™ Substrate (Catalog #: G649A)
Developer Reagent (Catalog #: G653)

Assay Protocol

The HDAC11 assay is performed using the HDAC-Glo I/II™ Activity Assay Kit (Promega, Cat# G6420), 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 HDAC11, 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 HDAC11.

Step 1. Thaw the Active HDAC11 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 HDAC11 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 Develper 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 HDAC11 (Catalog #H93-30G)

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 HDAC11 preparation. Replace the HDAC11 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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