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Catalog #	Aliquot Size
H89-31G-05	5 µg
H89-31G-10	10 µg

HDAC7, Active

Recombinant human protein expressed in Sf9 cells

Catalog # H89-31G

Lot # H200-1

Product Description

Recombinant human HDAC7 (501-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_015401.

Gene Aliases

HD7A; HDAC7A; DKFZp586J0917; FLJ99588

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.

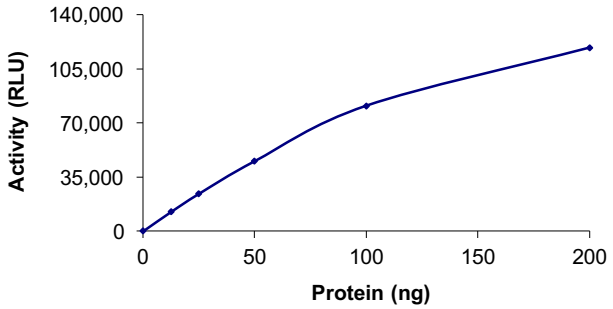
Scientific Background

HDAC7 or Histone deacetylase 7 belongs to the histone deacetylase/acuc/apha family and is a component of the histone deacetylase complex. The protein encoded by HDAC7 gene has sequence homology to members of the histone deacetylase family whose protein promotes repression mediated via the transcriptional co-repressor SMRT (1). HDAC7 interacts with β-catenin keeping endothelial cells in a low proliferation stage. HDAC7 regulates NUR77 and apoptosis in developing thymocytes (2). HDAC7 is expressed in human osteoarthritis that contributes to cartilage degradation via promoting MMP-13 gene expression.

References

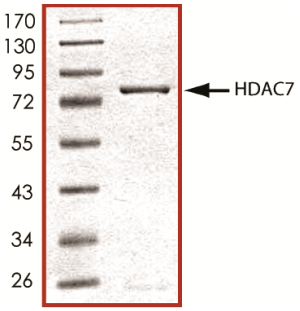
1. Kao, H. Y. et.al: Isolation of a novel histone deacetylase reveals that class I and class II deacetylases promote SMRT-mediated repression. Genes Dev. 14: 55-66, 2000.
2. Dequiedt, F. et.al: HDAC7, a thymus-specific class II histone deacetylase, regulates Nur77 transcription and TCR-mediated apoptosis. Immunity 18: 687-698, 2003.

Specific Activity



The specific activity of HDAC7 was determined to be **80 RLU/min/ng** as per activity assay protocol.

Purity



The purity of HDAC7 was determined to be **>90%** by densitometry.
Approx. MW **80kDa**.

HDAC7, Active

Recombinant human protein expressed in Sf9 cells

Catalog Number	H89-31G
Specific Activity	80 RLU/min/ng
Specific Lot Number	H200-1
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 HDAC7 (Catalog #: H89-31G)

Active HDAC7 (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 HDAC7 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 HDAC7 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 HDAC7, 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 HDAC7.

Step 1. Thaw the Active HDAC7 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 HDAC7 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 HDAC7 (Catalog #H89-31G)

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 HDAC7 preparation. Replace the HDAC7 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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