SignalChem
Specialists in Signaling Proteins
1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
H86-31G-05	5 µg
H86-31G-10	10 µg

HDAC4, Active

Recombinant human protein expressed in Sf9 cells

Catalog # H86-31G
Lot # T848-1

Product Description

Recombinant human HDAC4 (612-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_006037.

Gene Aliases

HA6116; HD4; HDAC-A; HDACA; KIAA0288

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.

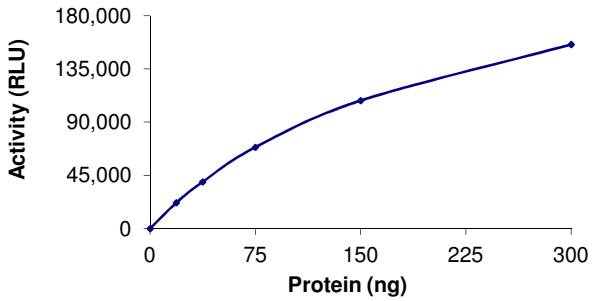
Scientific Background

HDAC4 or Histone deacetylase 4 belongs to class II of the histone deacetylase/acuc/apha family and is a component of the histone deacetylase complex that represses transcription when tethered to a promoter. HDAC4 does not bind DNA directly, but through transcription factors MEF2C and MEF2D. Binding of the N terminus of HDAC4 to MEF2C represses MEF2C transcription activity. The catalytic domain of HDAC4 interacts with HDAC3 via the transcriptional corepressor NCOR2. Suppression of HDAC4 binding to NCOR2 and to HDAC3 results in loss of enzymatic activity associated with HDAC4.

References

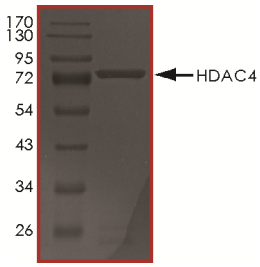
1. Wang, A. H. et al: HDAC4, a human histone deacetylase related to yeast HDA1, is a transcriptional corepressor. Molec. Cell. Biol. 19: 7816-7827, 1999.
2. Fischle, W. et al: Enzymatic activity associated with class II HDACs is dependent on a multiprotein complex containing HDAC3 and SMRT/N-CoR. Molec. Cell 9: 45-57, 2002.

Specific Activity



The specific activity of HDAC4 was determined to be **68 RLU/min/ng** as per activity assay protocol.

Purity



The purity of HDAC4 was determined to be **>95%** by densitometry.
Approx. MW **77kDa**.

HDAC4, Active

Recombinant human protein expressed in Sf9 cells

Catalog #	H86-31G
Specific Activity	68 RLU/min/ng
Lot #	T848-1
Purity	>95%
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
www.signalchem.com

Activity Assay Protocol

Reaction Components

Active HDAC4 (Catalog #: H86-31G)

Active HDAC4 (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 HDAC4 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 HDAC4 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 HDAC4, 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 HDAC4.

Step 1. Thaw the Active HDAC4 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 HDAC4 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 10ml 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 HDAC4 (Catalog #H86-31G)

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 HDAC4 preparation. Replace the HDAC4 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)

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com
www.signalchem.com