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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
H84-30G-05	5 µg
H84-30G-10	10 µg

HDAC2, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # H84-30G

Lot # C2078-6

Product Description

Full-length recombinant human HDAC2 was expressed by baculovirus in Sf9 insect cells using a C-terminal GST tag. The gene accession number is NM_001527.

Gene Aliases

RPD3; YAF1

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.

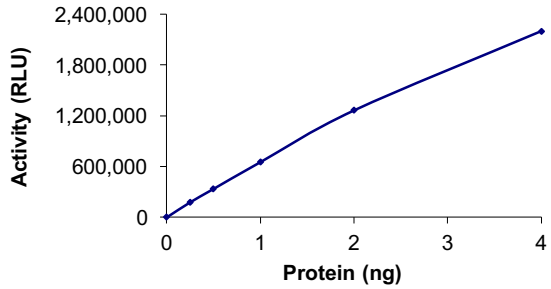
Scientific Background

HDAC2 or Histone deacetylase 2 belongs to the histone deacetylase family that acts via the formation of large multiprotein complexes, and is responsible for the deacetylation of lysine residues at the N-terminal regions of core histones (H2A, H2B, H3 and H4). HDAC2 forms transcriptional repressor complexes by associating with many different proteins and plays an important role in transcriptional regulation, cell cycle progression and developmental events. HDAC2 functions in modulating synaptic plasticity and long-lasting changes of neural circuits, which in turn negatively regulates learning and memory (1). HDAC1 and HDAC2 are functionally redundant in cardiac growth and development and they maintain cardiomyocyte identity and function (2).

References

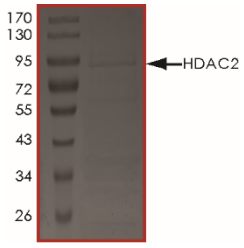
1. Guan, J.S.. et.al: HDAC2 negatively regulates memory formation and synaptic plasticity. Nature 459: 55-60, 2009.
2. Montgomery, R. L. et.al: Histone deacetylases 1 and 2 redundantly regulate cardiac morphogenesis, growth, and contractility. Genes Dev. 21: 1790-1802, 2007.

Specific Activity



The specific activity of HDAC2 was determined to be **4,200 RLU/min/ng** as per activity assay protocol.

Purity



The purity of HDAC2 was determined to be **~70%** by densitometry.
Approx. MW **92kDa**.

HDAC2, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog	H84-30G
Specific Activity	4,200 RLU/min/ng
Lot	C2078-6
Purity	~70%
Concentration	0.05µg/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 HDAC2 (Catalog #: H84-30G)

Active HDAC2 (0.05µg/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 HDAC2 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 HDAC2 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 HDAC2, 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 HDAC2.

Step 1. Thaw the Active HDAC2 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 HDAC2 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 HDAC2 (Catalog #H84-30G)

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 HDAC2 preparation. Replace the HDAC2 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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