

HDAC6, Active

Recombinant full-length protein expressed in Sf9 cells

Catalog # H88-30G

Lot # J3765-7

Product Description

Recombinant full-length human HDAC6 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is <u>BC069243</u>.

Gene Aliases

HD6; JM21; KIAA0901

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

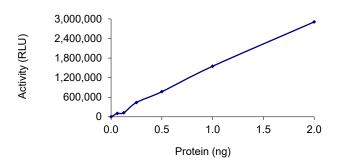
HDAC6 or Histone deacetylase 6 belongs to the histone deacetylase/acuc/apha family and is a component of the histone deacetylase complex. Histones play a critical role in transcriptional regulation, cell cycle progression, and developmental events. The protein encoded by this gene belongs to class II of the histone deacetylase family that regulates important biologic processes beyond histone metabolism and gene transcription (1). HDAC6 is a central component of the stress response that regulates SG formation and potentially contributes to control of RNA metabolism and translation (2).

References

- 1. Hubbert, C. et.al: HDAC6 is a microtubule-associated deacetylase. Nature 417: 455-458, 2002.
- 2. Kwon, S. et.al: The deacetylase HDAC6 is a novel critical component of stress granules involved in the stress response. Genes Dev. 21: 3381-3394, 2007.

| Catalog # | Aliquot Size |
|-------------|--------------|
| H88-30G -05 | 5 µg |
| H88-30G -10 | 10 µg |

Specific Activity



The specific activity of HDAC6 was determined to be **5,934 RLU** /min/ng as per activity assay protocol.

Purity



The purity of HDAC6 was determined to be >85% by densitometry, approx. MW 180 kDa.

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Recombinant full-length human protein expressed in Sf9 cells

| Catalog # | H88-30G |
|--------------------|--|
| Specific Activity | 5,934 RLU/min/ng |
| Lot # | J3765-7 |
| Purity | >85% |
| Concentration | 0.1 μg/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 HDAC6 (Catalog #: H88-30G)

Active HDAC6 (0.1µg/µl) diluted with HDAC-Glo I/IITM 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 HDAC6 for optimal results).

HDAC-Glo I/II[™] Activity Assay Kit (Promega)

HDAC-Glo I/IITM Buffer, 25ml HDAC-Glo I/IITM Substrate Cake, 1 bottle HDAC-Glo I/IITM Developer Reagent, 10µl

Assay Protocol

The HDAC6 assay is performed using the HDAC-Glo I/IITM 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 HDAC6, 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 HDAC6.

- Step 1. Thaw the Active HDAC6 and HDAC-Glo I/II[™] Developer Reagent on ice.
- Step 2. Thaw the HDAC-Glo I/IITM Buffer and HDAC-Glo I/IITM Substrate and equilibrate to room temperature.
- Step 3. Prepare the following working solutions:
 - Diluted active HDAC6 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 HDAC6 (Catalog #H88-30G)

- Component 2. 20µl of HDAC-Glo I/IITM Reaction Reagent in step 3
- Step 5. Set up a blank control as outlined in step 4 by excluding the addition of the diluted HDAC6 preparation. Replace the HDAC6 preparation with an equal volume of HDAC-Glo I/II[™] Buffer.
- Step 6. Incubate the mixture at room temperature for 30 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)

SAFETY DATA SHEET

Product Name: HDAC6, Active

Catalog # H88-30G

This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.

Manufacturer's Name: Street Address: City, Prov. Postal Code: Fax: EMERGENCY PHONE: SignalChem Biotech Inc. 110-13120 Vanier Place Richmond, BC, V6V 2J2 604-232-4601 604-232-4600

Article 2 - Hazard Identification

- WHMIS Classification: Not WHMIS controlled.
- GHS classification: Skin irritation (Category 3); Eye irritation (Category 2B).
- Hazard Pictograms: none.
- Signal words: Warning.
- Hazard statements: Causes mild skin irritation (H316); Causes eye irritation (H320).
- Precautionary statements: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. (P305 + P351 + P338).
- Other hazards: none known.

Article 3 – Composition/Information on Ingredients

Chemical Characterization: Mixtures. Description: This product consists of the substances listed below.

| Common name | Chemical name | CAS-No. | Concentration |
|--|--|-------------------|---------------|
| Glycerol | Glycerol | 56-81-5 | ≤25% |
| NaCl | Sodium chloride | 7647-14-5 | ≤1.753 % |
| Tris-HCl; Tris (hydroxymethyl) aminomethane hydrochloride | 2 – Amino – 2 - (hydroxymethyl) propane - 1, 3 - diol hydrochloride | 1185-53-1 | <0.8% |
| Glutathione | Glutathione | 70-18-8 | 0.307% |
| Protein | | No data available | ≤0.02% |
| DTT; Dithiothreitol | (R*,R*)-1,4-Dimercaptobutane-2,3-diol | 3483-12-3 | 0.0038% |
| EDTA | Ethylenediaminetetraacetic acid | 6381-92-6 | 0.0037% |
| PMSF; Phenylmethanesulfonyl fluoride | a-Toluenesulphonyl fluoride | 329-98-6 | 0.002% |

Article 4 – First-aid Measures

- General information: Consult a physician by providing the SDS.
- After inhalation: Breathe in fresh air. If cannot breathe, give artificial respiration and consult a physician.
- After skin contact: Immediately wash with soap and plenty of water and rinse thoroughly. Consult a physician.
- After eye contact: Rinse opened eyes with plenty of water for at least 15 minutes. Consult a physician.
- After swallowing: rinse the mouth with plenty of water and consult a physician.

Article 5 - Fire-fighting Measures

- Suitable extinguishing media: Use water spray, extinguishing powder, carbon dioxide, or other appropriate measure that is suitable to the environment.
- Specific hazards arising from the substance or mixture: None known.
- Special protective equipment and precautions for fire-fighters: Self-contained breathing apparatus if necessary.

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Article 6 – Accidental Release Measures

- Personal precautions, protective equipment and emergency procedures: Apply standard laboratory practices and personal protective equipment. Avoid breathing vapors, mist, or gas. Ensure adequate ventilation.
- Environmental precautions: Do not allow to enter drains.
- Methods and materials for containment and cleaning up: Absorb on sand or vermiculite and place in closed containers for disposal.

Article 7 - Handling and Storage

- Precautions for sate handling: Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.
- Conditions for safe storage: Store in a dry and well-ventilated place in -70 °C. Keep container upright and tightly closed.

Article 8 - Exposure Controls/Personal Protection

 Components with limit monitoring values at workplace: Glycerol (CAS-No: 56-81-5)

| , | | |
|--------|---|--------------------------|
| Values | Control parameters | Regulations |
| TWA | 10 mg/m ³ for mist | British Columbia, Canada |
| TWA | 3 mg/m ³ for respirable mist | British Columbia, Canada |
| TWA | 10 mg/m ³ | Alberta, Canada |
| TWAEV | 10 mg/m ³ | Ontario, Canada |
| TWAEV | 10 mg/m ³ | Quebec, Canada |
| TWA | 10 mg/m ³ | USA |

Appropriate engineering controls:

Apply adequate ventilation including mechanical exhaust or laboratory fume hood. Follow standard laboratory practices.

- Individual protection measures:
- Respiratory protection:

Use appropriate respirator if there is inadequate ventilation by following the government standards.

Hand protection:

Wear gloves and use proper glove removal technique to avoid skin contact. Discard gloves after use by following the applicable laboratory regulations. Wash and dry hands.

Eve/face protection:

Safety goggles with side-shields approved under appropriate government standards.

Skin/body protection:

Use appropriate clothing, footwear and any additional protection measures to protect from splashing or contamination.

Article 9 – Physical and Chemical Properties

| Appearance: Colorless fluid. | Danger of explosion: Product does not present an explosion hazard. |
|--|--|
| Odour/Odour Threshold: Not determined. | Explosion limits: Lower: 0.9 Vol %; Upper: 0.0 Vol %. |
| pH: Not available. | Decomposition temperature: Not available. |
| Melting point/freezing point: Not determined. | Vapor pressure at 20 °C: 0.1 hPa |
| Boiling point/Boiling range: 100 °C. | Density: Not determined. |
| Flash point: > 100 °C. | Relative density: Not determined. |
| Flammability (solid, gaseous): Not determined. | Vapor density: Not determined. |
| Ignition temperature: 400 °C. | Evaporation rate: Not determined. |
| Auto-igniting: Product is not self-igniting. | Solubility in / Miscibility with Water: Fully miscible. |

Article 10 - Stability and Reactivity

- Reactivity: Stable under recommended transport and storage conditions.
- Chemical stability: Stable under recommended transport and storage conditions.
- Possible hazardous reactions: No dangerous reactions known.
- Conditions to avoid: Heat and moisture.
- Incompatible materials: Strong acids/bases, strong oxidizing/reducing agents.
- Hazardous decomposition products: Carbon oxides may formed under fire conditions; no known decomposition information for other decomposition products.

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and Reactivity

FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.

SAFETY DATA SHEET

Article 11 - Toxicological Information

- Acute toxicity: Not available.
- LD/LC50: Not available.
- Skin corrosion/irritation: Not available.
- Serious eye damage/eye irritation: Not available.
- Respiratory or skin sensitization: Not available.
- Germ cell mutagenicity: Not available.
- Carcinogenicity: No components are listed in IARC, or NTP, or OSHA, or ACGIH.
- Reproductive toxicity: Not available.
- Teratogenicity: Not available.
- Specific target organ toxicity single exposure/ repeated exposure (GHS): Not available.
- Aspiration hazard: Not available.
- Potential health effects: Inhalation: May be harmful if inhaled. May cause respiratory tract irritation. Ingestion: May be harmful if swallowed.
 Skin: May be harmful if absorbed through skin. May cause skin irritation.
 Eyes: May cause eye irritation.
- Signs and Symptoms of Exposure:
- Prolonged or repeated exposure can cause: Nausea, Dizziness.
- Synergistic effects: Not available.

Article 12 - Ecological Information

- Eco-toxicity: Not applicable.
- Biodegradability: Not applicable.
- Bio-accumulative potential: Not applicable.
- Mobility in soil: Not applicable.
- PBT and vPvB assessment: Not applicable.
- Other adverse effects: Not applicable.

Article 13 - Disposal Considerations

- Disposal methods: In accordance to applicable national, regional, or local laws and regulations. For additional handling information and protection of employees please refer to Article 7 and 8.
- Contaminated packaging: Disposal should be made in accordance to official regulations. Use water or cleansing agents to clean the area.

Article 14 - Transport Information

- DOT: Not dangerous goods.
- IMDG: Not dangerous goods.
- IATA: Not dangerous goods.

Article 15 – Regulatory Information

- WHMIS Classification: Non-hazardous.
- GHS label elements: Not applicable.
- Signal word: Not applicable.
- Hazard statements: Not applicable.

Article 16 - Other Information

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. SignalChem shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalog for additional terms and conditions of sale.