Catalog #	Aliquot Size
S40-31H-05	5 µg
S40-31H-10	10 µg

SIRT6, Active

Recombinant human protein expressed in Sf9 cells

Catalog # S40-31H

Lot # Z1156-3

Product Description

Recombinant human SIRT6 (23-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal His tag. The gene accession number is NM_016539.

Alternative Name(s)

SIR2L6

Formulation

Recombinant protein stored in 50mM sodium phosphate, pH 7.0, 300mM NaCl, 150mM imidazole, 0.1mM PMSF, 0.25mM DTT, 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.

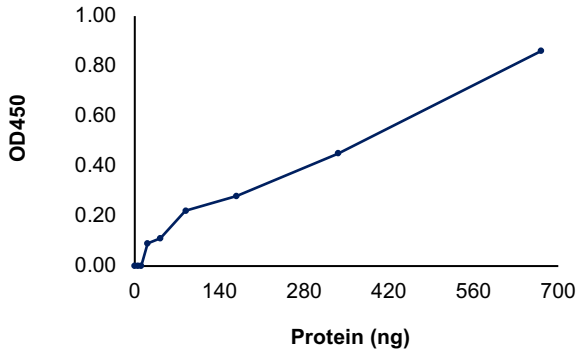
Scientific Background

SIRT6 is a member of the sirtuin family of proteins which are homologs to the yeast Sir2 protein. Sirtuin family contain a sirtuin core domain and are grouped into four classes with SIRT6 being a member of class IV. Human SIRT6 protein is a NAD(+)-dependent histone H3 lysine-9 deacetylase that modulates telomeric chromatin (1). SIRT6 associates specifically with telomeres and SIRT6 depletion leads to telomere dysfunction with end-to-end chromosomal fusions and premature cellular senescence. SIRT6 -/- mouse cells show that SIRT6 promotes resistance to DNA damage and suppresses genomic instability in association with a role in base excision repair (2).

References

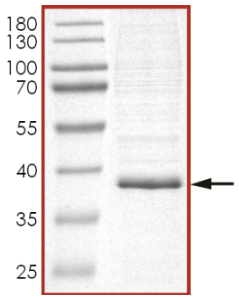
1. Michishita, E. et al: SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin. Nature 452: 492-496, 2008.
2. Mostoslavsky, R. et al: Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. Cell 124: 315-329, 2006.

Specific Activity



The specific activity of SIRT6 was determined to be **318 ng deacetylated HH3/min/mg** as per activity assay protocol.
Note that this activity protocol and specific activity units have been revised compared to previous lots of this product.

Purity



The purity of SIRT6 was determined to be **>85%** by densitometry. Approx. MW **39kDa**.

SIRT6, Active

Recombinant human protein expressed in Sf9 cells

Catalog #	S40-31H
Specific Activity	318 ng deacetylated HH3/min/mg
Lot #	Z1156-3
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 SIRT6 (Catalog #: S40-31H)

Active SIRT6 (0.1µg/µl) diluted with Acetyl-transferase Dilution Buffer (Catalog #: A21-09) 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 SIRT6 for optimal results).

Acetyltransferase Dilution Buffer (Cat. #: A21-09)

Acetyltransferase Assay Buffer (Catalog #: A01-09) diluted at a 1:4 ratio (5X dilution) with 50 ng/µl BSA solution.

Acetyltransferase Assay Buffer (Cat. #: A01-09)

Buffer components: 250mM Tris-HCl, pH 8.0, 0.5mM EDTA, 25% glycerol. Add 2mM DTT to Acetyltransferase Assay Buffer prior to use.

NAD solution

Nicotinamide adenine dinucleotide, NAD (Biosynth, Catalog#: NN11969) dissolved in H_2O to make a stock solution at a final concentration of 500mM. The working concentration is 0.5mM.

Substrate (Catalog#: H12-358)

Acetylated Histone H3 Peptide (1-21) diluted in PBS to a final concentration of 0.2 µg/ml.

Standard

Acetylated Histone H3 Peptide (1-21) (Catalog #: H12-358) may be used as a standard.

Acetyl Lysine Detection Antibody, rabbit polyclonal, HRP conjugated (Catalog#: L95-66DR)

Dilute the detection antibody at 1000-fold with 1% BSA (equivalent to 0.25 µg/mL) prior to use. TMB substrate was from BD Biosciences (Catalog#: 555214)

Assay Protocol

Step 1. Dilute Acetylated Histone H3 Peptide (1-21) (**Substrates**) in PBS to 0.2 µg/ml. Dilute Acetylated Histone H3 Peptide to 2 µg/ml (**Standard**), then make 3-fold dilution for 8 dilutions (standard). Add 50µl/well of the Acetylated Histone H3 Peptide to 94 wells of a 96 well ELISA plate, leaving 2 wells without coating as **Blank** control. Incubate in 4°C overnight.

Step 2. Discard solution. Wash plate 3 times with ELISA Wash buffer (1X PBS with 0.05% Tween-20).

Step 3. Block plate with 200µl/well of 1% BSA in PBS (**Blocking Buffer**). Incubate at room temperature for two hours.

Step 4. Wash the plate by repeating Step 2.

Step 5. Thaw the Active SIRT6, Acetyltransferase Assay Buffer, and Acetyltransferase Dilution Buffer on ice.

Step 6. Dilute Active SIRT6 with Activity Dilution Buffer. Leave on ice.

Step 7. Add the following reaction components to substrate coated wells, bringing the initial reaction volume up to 40µl.

- **Component 1.** 10µl of diluted Active SIRT6 (Catalog #S40-31H)
- **Component 2.** 30µl of Acetyltransferase Assay Buffer (Catalog #: A01-09)

Step 8. Add 40µl of Acetyltransferase Dilution Buffer to **Standard** wells and **Blank** control wells.

Step 9. Initiate the reaction by the addition 10µl of 2.5 mM NAD solution bringing the final volume up to 50µl, and a final concentration of NAD of 0.5 mM. Incubate the mixture at 37°C for 1 hour.

Step 10. Wash the plate by repeating step 2.

Step 11. Dilute HRP conjugated anti-acetyl lysin antibody to 0.25 µg/ml in PBS with 1% BSA. Add 50µl/well and incubate at room temperature for 30 minutes.

Step 12. Discard solution and wash plate 6 times with ELISA Wash buffer.

Step 13. Add 50µl/well of TMB Substrate and incubate approximately 10 minutes for blue color development.

Step 14. Stop the reaction by adding 50µl/well of 2M sulfuric acid, turning the blue color to yellow.

Step 15. Read the absorbance in microplate reader at 450 nm and 570 nm.

Step 16. Calculate average duplicate readings for sample wells, standard wells and blank wells, if required.

Step 17. Generate a standard curve as OD value versus amount of standard at each concentration point. Then calculate the corresponding ng of acetylated product from the standard curve.

Step 18. Subtract the total acetylated product (SIRT at 0 ng/well concentration) with corresponding acetylated product for deacetylated product value (ng)

Step 19. Calculate the SIRT specific activity as outlined below.

SIRT Deacetylation Activity (ng/min/mg)

SIRT Activity (ng/min/mg) = Deacetylated product (ng) / (Reaction time in min) * (Enzyme amount in mg)

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SAFETY DATA SHEET

Article 1 – Product Identification

Product Name: SIRT6, Active **Catalog # S40-31H**

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:	SignalChem Biotech Inc.
Street Address:	*110-13120 Vanier Place*
City, Prov. Postal Code:	*Richmond, BC, V6V 2J2*
Fax:	604-232-4601
EMERGENCY PHONE:	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	1.75%
Imidazole	1,3-Diaza-2,4-cyclopentadiene	288-32-4	≤1.02%
Sodium Phosphate, Dibasic	Sodium Phosphate, Dibasic	7782-85-6	1.34%
Protein		No data available	≤0.02%
DTT; Dithiothreitol	(R*,R*)-1,4-Dimercaptobutane-2,3-diol	3483-12-3	0.0038%
PMSF; Phenylmethanesulfonyl fluoride	α-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 breath, 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.

SAFETY DATA SHEET

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/m³ for mist	British Columbia, Canada
TWA	3 mg/m³ for respirable mist	British Columbia, Canada
TWA	10 mg/m³	Alberta, Canada
TWAEV	10 mg/m³	Ontario, Canada
TWAEV	10 mg/m³	Quebec, Canada
TWA	10 mg/m³	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.

Eye/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.

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.