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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 # E300-1

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.

Gene Aliases

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.

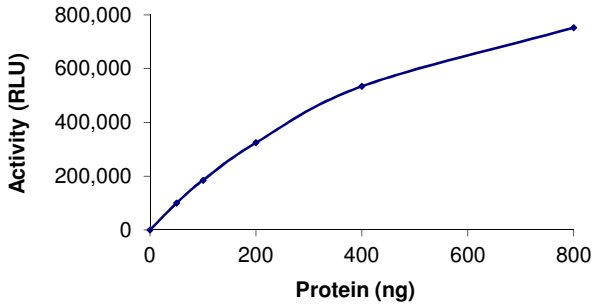
Scientific Background

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

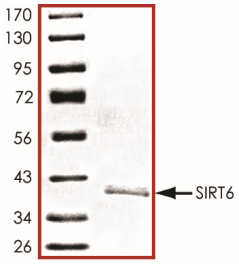
1. Michishita, E. et al: SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin. Nature 452: 492-496, 2008.
2. Mostoslavsky, R. et al: Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. Cell 124: 315-329, 2006.

Specific Activity



The specific activity of SIRT6 was determined to be **120 RLU/min/ng** as per activity assay protocol.

Purity



The purity of SIRT6 was determined to be **>90%** by densitometry. Approx. MW **39kDa**.

SIRT6, Active

Recombinant human protein expressed in Sf9 cells

Catalog #	S40-31H
Specific Activity	120 RLU/min/ng
Lot #	E300-1
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 SIRT6 (Catalog #: S40-31H)

Active SIRT6 (0.1µg/µl) diluted with SIRT-Glo™ 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 SIRT6 for optimal results).

SIRT-Glo™ Activity Assay Kit (Promega)

SIRT-Glo™ Buffer, 25ml
SIRT-Glo™ Substrate Cake, 1 bottle
SIRT-Glo™ Developer Reagent, 10µl

Assay Protocol

The SIRT6 assay is performed using the SIRT-Glo™ Activity Assay Kit (Promega), which is designed for assaying SIRTs, the NAD+ dependent, histone deacetylase class III enzymes. The Activity Assay Kit examines sequential reaction of deacetylation of an acetylated luminogenic peptide substrate by SIRT6, 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 SIRT6.

Step 1. Thaw the Active SIRT6 and SIRT-Glo™ Developer Reagent on ice.

Step 2. Thaw the SIRT-Glo™ Buffer and SIRT-Glo™ Substrate and equilibrate to room temperature.

Step 3. Prepare the following working solutions:

- Diluted active SIRT6 with SIRT-Glo™ Buffer on ice
- Prepare the SIRT-Glo™ Substrate Solution by adding 10ml of SIRT-Glo™ Buffer to the SIRT-Glo™ Substrate Cake bottle. (The aliquots can be refrozen if developer reagent has not been added).
- Prepare the SIRT-Glo™ 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 SIRT6 (Catalog #S40-31H)

Component 2. 20µl of SIRT-Glo™ Reaction Reagent in step 3

Step 5. Set up a blank control as outlined in step 4 by excluding the addition of the diluted SIRT6 preparation. Replace the SIRT6 preparation with an equal volume of SIRT-Glo™ 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 SIRT specific activity as outlined below.

SIRT Specific Activity (SA) (RLU/min/ng)

Corrected RLU from reaction / (Reaction time in min)*(Enzyme amount in ng)

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