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Catalog #	Aliquot Size
S35-31G-05	5 µg
S35-31G-10	10 µg

SIRT1, Active

Recombinant human protein expressed in Sf9 cells

Catalog # S35-31G
Lot # H2930-3

Product Description

Recombinant human SIRT1 (193-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_012238.

Gene Aliases

SIR2L1

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, and 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.

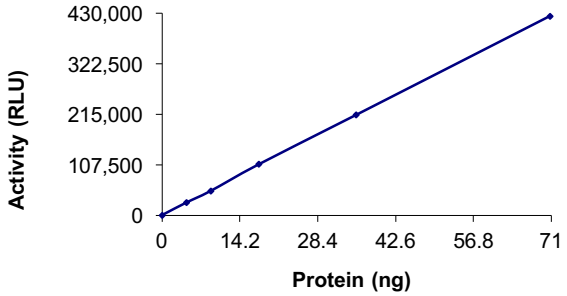
Scientific Background

SIRT1 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 SIRT1 being a member of class I. SIRT1 is a stress-response and chromatin-silencing factor (1). It is an NAD (+)-dependent histone deacetylase involved in various nuclear events such as transcription, DNA replication, and DNA repair. SIRT1 protein binds and deacetylates the p53 protein (2). Expression of wild type SIRT1 in human cells reduces the transcriptional activity of p53 indicating that SIRT1 is involved in the regulation of p53 function via deacetylation.

References

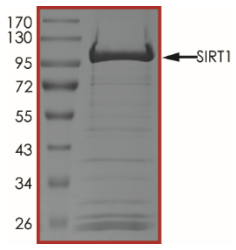
1. Tanny, J. C. et al: An enzymatic activity in the yeast Sir2 protein that is essential for gene silencing. Cell 99: 735-745, 1999.
2. Vaziri, H. et al: hSIR2-SIRT1 functions as an NAD-dependent p53 deacetylase. Cell 107: 149-159, 2001.

Specific Activity



The specific activity of SIRT1 was determined to be **1690 RLU/min/ng** as per activity assay protocol.

Purity



The purity of SIRT1 was determined to be **>75%** by densitometry. Approx. MW **110 kDa**.

SIRT1, Active

Recombinant human protein expressed in Sf9 cells

Catalog #	S35-31G
Specific Activity	1690 RLU/min/ng
Lot #	H2930-3
Purity	>75%
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 SIRT1 (Catalog #: S35-31G)

Active SIRT1 (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 SIRT1 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 SIRT1 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 SIRT1, 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 SIRT1.

Step 1. Thaw the Active SIRT1 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 SIRT1 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 SIRT1 (Catalog #S35-31G)

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 SIRT2 preparation. Replace the SIRT1 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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