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1-866-9 KINASE (54-6273)

Catalogue #	Aliquot Size
S37-38G -05	5 µg
S37-38G -10	10 µg

SIRT3, Active

Recombinant human protein expressed in Sf9 cells

Catalog # S37-38G
Lot # S299-5

Product Description

Recombinant human SIRT3 (47-end) was expressed by baculovirus in Sf9 insect cells using a C-terminal GST tag. The gene accession number is NM_012239.

Gene Aliases

SIR2L3

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.

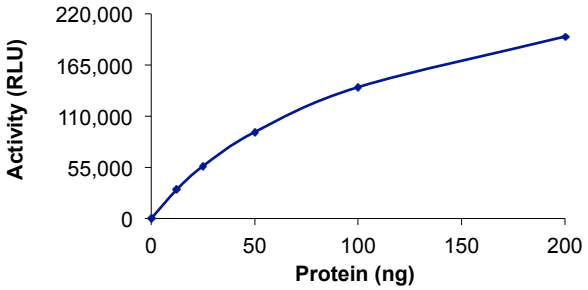
Scientific Background

SIRT3 is a member of the class I of the Sirtuin family that is characterized by a Sirtuin core domain. Sirtuin are grouped into four classes and are homologs to the Sir2 protein. SIRT3 is known to regulate the epigenetic gene silencing and suppresses recombination of rDNA (1). SIRT3 may function as an intracellular regulatory protein with mono-ADP-ribosyltransferase activity that is essential for its silencing function. SIRT3 regulates and maintains basal ATP levels in the cell (2). SIRT3 also modulates mitochondrial intermediary metabolism and fatty acid use during fasting (3).

References

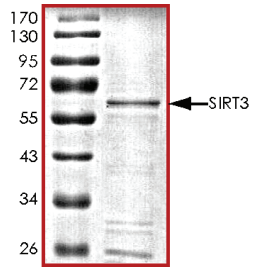
1. Tanny, J. C. et.al: An enzymatic activity in the yeast Sir2 protein that is essential for gene silencing. Cell 99: 735-745, 1999.
2. Ahn, B.-H. et.al: A role for the mitochondrial deacetylase Sirt3 in regulating energy homeostasis. Proc. Nat. Acad. Sci. 105: 14447-14452, 2008.
3. Hirschey, M. D. et.al: SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. Nature 464: 121-125, 2010.

Specific Activity



The specific activity of SIRT3 was determined to be **145 RLU /min/mg** as per activity assay protocol.

Purity



The purity of SIRT3 was determined to be **>70%** by densitometry, approx. MW **66kDa**.

SIRT3, Active

Recombinant human protein expressed in Sf9 cells

Catalog Number	S37-38G
Specific Activity	145 RLU/min/mg
Specific Lot Number	S299-5
Purity	>70%
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 SIRT3 (Catalog #: S37-38G)

Active SIRT3 (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 SIRT3 for optimal results).

SIRT-Glo™ Activity Assay Kit (Promega, Catalog #: G6450)

SIRT-Glo™ Buffer, 25ml (Catalog #: G658A)
SIRT-Glo™ Substrate Cake, 1 vial (Catalog #: G644A)
SIRT-Glo™ Developer Reagent, 10μl (Catalog #: G653A)

Assay Protocol

The SIRT3 assay is performed using the SIRT-Glo™ Activity Assay Kit (Promega; Catalog #: G6450), 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 SIRT3, 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 SIRT3.

Step 1. Thaw the Active SIRT3 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 SIRT3 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 Develper 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 SIRT3 (Catalog #S37-38G)

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 SIRT3 preparation. Replace the SIRT3 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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