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Catalogue #	Aliquot Size
S41-30H-05	5 µg
S41-30H-10	10 µg
S41-30H-20	20 µg

SIRT7, Active

Full-length recombinant human protein expressed in Sf9 cells

Catalog # S41-30H
Lot # E312-1

Product Description

Full-length recombinant human SIRT7 was expressed by baculovirus in Sf9 insect cells using an N-terminal His tag. The gene accession number is NM_016538.

Gene Aliases

SIR2L7, MGC126840, MGC126842

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.

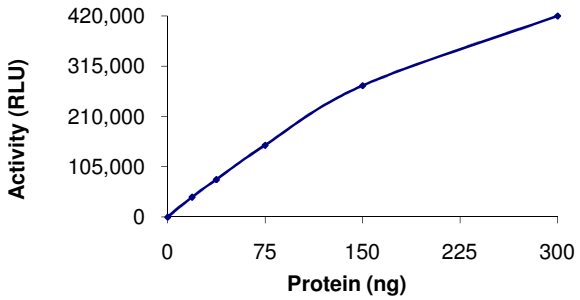
Scientific Background

SIRT7 is a member of the class IV of sirtuin family of proteins which are homologs to the yeast Sir2 protein and play a role in cell differentiation, proliferation, apoptosis, metabolism, and senescence. SIRT7 associates with active rRNA genes and histones. Overexpression of SIRT7 increases pol I-mediated transcription whereas knockdown of SIRT7 or inhibition of its catalytic activity results in decreased association of pol I with rDNA and reduced pol I transcription (1). Depletion of SIRT7 stops cell proliferation and triggers apoptosis. SIRT7 deacetylates p53 and increases cellular resistance to cytotoxic and oxidative stress (2).

References

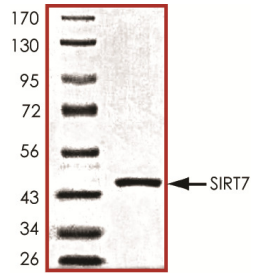
1. Ford, E. et al: Mammalian Sir2 homolog SIRT7 is an activator of RNA polymerase I transcription. Genes Dev. 20: 1075-1080, 2006.
2. Vakhrusheva, O. et al Sirt7 increases stress resistance of cardiomyocytes and prevents apoptosis and inflammatory cardiomyopathy in mice. Circ. Res. 102: 703-710, 2008.

Specific Activity



The specific activity of SIRT7 was determined to be **140 RLU/min/ng** as per activity assay protocol.

Purity



The purity of SIRT7 was determined to be **>95%** by densitometry. Approx. MW **46kDa**.

SIRT7, Active

Full-length recombinant human protein expressed in Sf9 cells

Catalog Number	S41-30H
Specific Activity	140 RLU/min/ng
Specific Lot Number	E312-1
Purity	>95%
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 SIRT7 (Catalog #: S41-30H)

Active SIRT7 (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 SIRT7 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 SIRT7 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 SIRT7, 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 SIRT7.

Step 1. Thaw the Active SIRT7 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 SIRT7 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 SIRT7 (Catalog #S41-30H)

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 SIRT7 preparation. Replace the SIRT7 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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