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1-866-9 KINASE (54-6273)

| Catalogue # | Aliquot Size |
|---|---|
| S39-30H-05 | 5 µg |
| S39-30H-10 | 10 µg |

# SIRT5, Active

Recombinant full length protein expressed in Sf9 cells

**Catalog # S39-30H**
*Lot # E297-1*

## Product Description

Recombinant full length human SIRT5 was expressed by baculovirus in Sf9 insect cells using an N-terminal His tag. The gene accession number is NM_012241.

## Gene Aliases

SIR2L5

## 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.

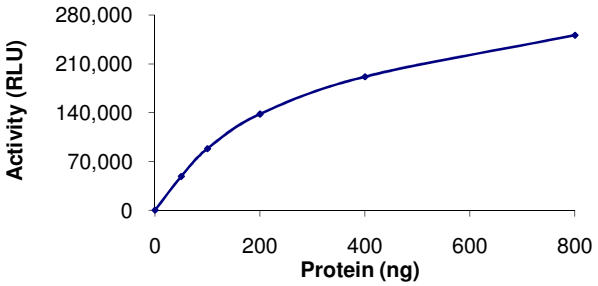
## Scientific Background

SIRT5 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 SIRT5 being a member of class III. SIRT5 consists of eight exons and is found in two isoforms which encode a 310 aa and a 299 aa protein, respectively. Human SIRT5 is most predominantly expressed in heart muscle cells and in lymphoblasts. Fluorescence in situ hybridization analysis localized the human SIRT5 gene to chromosome 6p23. SIRT5 can deacetylate cytochrome c, a protein of the mitochondrial intermembrane space with a central function in oxidative metabolism as well as apoptosis initiation (1).

## References

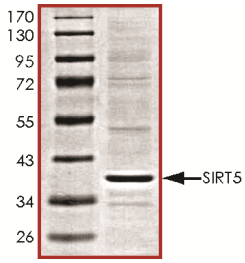

1. Schlicker C. et al: Substrates and regulation mechanisms for the human mitochondrial sirtuins Sirt3 and Sirt5. J Mol Biol. 2008 Oct 10;382(3):790-801.
2. Mahlknecht, U. et al: Assignment of the NAD-dependent deacetylase sirtuin 5 gene (SIRT5) to human chromosome band 6p23 by in situ hybridization. Cytogenet Genome Res. 2006;112(3-4):208-12.


## Specific Activity




The specific activity of SIRT5 was determined to be **23 RLU/min/ng** as per activity assay protocol.

## Purity




The purity of SIRT5 was determined to be **>75%** by densitometry. Approx. MW **39kDa**.


# SIRT5, Active

Recombinant full length protein expressed in Sf9 cells


| | |
|---|---|
| Catalog Number | S39-30H |
| Specific Activity | 23 RLU/min/ng |
| Specific Lot Number | E297-1 |
| 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 SIRT5** (Catalog #: S39-30H)

Active SIRT5 (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 SIRT5 for optimal results).

**SIRT-Glo™ Activity Assay Kit (Promega, Cat #G6450)**

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

## Assay Protocol

The SIRT5 assay is performed using the SIRT-Glo™ Activity Assay Kit (Promega; Cat# 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 SIRT5, 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 SIRT5.

**Step 1.** Thaw the Active SIRT5 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 SIRT5 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 SIRT5 (Catalog #S39-30H)

**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 SIRT5 preparation. Replace the SIRT5 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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