Recombinant Human Sirtuin 1/SIRT1

Catalog Number: 7714-DA

DESCRIPTION

Source	*E. coli*-derived human Sirtuin 1/SIRT1 protein Met1-Ser747, with a C-terminal 6-His tag. Accession # Q96EB6
N-terminal Sequence Analysis	Ala2
Predicted Molecular Mass	82 kDa

SPECIFICATIONS

SDS-PAGE	110-113 kDa, reducing conditions
Activity	Measured by its ability to remove the acetyl group from a fluorogenic peptide substrate Ac-RGK(Ac)-AMC (Catalog # ES016) in a coupled assay. The specific activity is >15 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>75%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl, DTT and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 1 mM DTT, pH 9.0
- Stop Buffer: 50 mM Tris, 100 mM NaCl, 30% (v/v) isopropanol, pH 8.0
- Recombinant Human Sirtuin 1/SIRT1 (rhSIRT1) (Catalog # 7714-DA)
- Fluorogenic Peptide Substrate Ac-Arg-Gly-Lys(Ac)-AMC (Catalog # ES016)
- β-nicotinamide adenine dinucleotide hydrate (β-NAD) (Sigma, Catalog # N6522), 100 mM in diH_2O
- Recombinant Mouse Active Trypsin 3/PRSS3 (Trypsin 3) (Catalog # 7235-SE)
- Nicotinamide (Sigma, Catalog # 72340) 100 mM in diH_2O
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhSIRT1 to 10 µg/mL in Assay Buffer.
2. Dilute Substrate to 500 µM in Assay Buffer containing 2 mM β-NAD.
3. Load 25 µL of 10 µg/mL rhSIRT1 into the plate in triplicate and start the reaction by adding 25 µL of 500 µM Substrate. Include a control containing 25 µL of 10 ng/µL rhSIRT1 only.
4. Incubate sealed plate at 37 °C for 30 minutes.
5. Prepare Stop Solution by combining Trypsin 3 and Nicotinamide in Stop Buffer to final concentrations of 0.2 µg/mL and 4 mM, respectively.
6. Add 50 µL of Stop Solution to all wells. Add 25 µL of 500 µM Substrate to the enzyme control wells after the addition of Stop Solution.
7. Seal plate tightly with plate sealer and incubate at room temperature for 15 minutes.
8. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in endpoint mode.

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted Fluorescence* (RFU) x Conversion Factor** (pmol/RFU)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Adjusted for Control

**Derived using calibration standard 7-amino, 4-Methyl Coumarin (AMC) (Sigma, Catalog # A9801).

Final Assay Conditions

Per Well:

- rhSIRT1: 0.25 µg
- Substrate: 125 µM

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -70 °C as supplied. • 3 months, -70 °C under sterile conditions after opening.

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BACKGROUND

SIRT1 is an NAD^+-dependent class III histone deacetylase. It is a member of the sirtuin family of proteins, homologous to the yeast Sir2 protein. It modulates intracellular activities through transcription regulation and functions in several separate cellular functions. SIRT1 deacetylates and inactivates tumor suppressor protein p53, a process regulated by Hypermethylated in Cancer 1 (HIC1) protein to allow cells to bypass apoptosis and survive DNA damage (1, 2). The level of SIRT1 is significantly elevated in mouse and human prostate cancer (3). SIRT1 promotes vascular relaxation by activating endothelial nitric oxide synthase (4). SIRT1 also protects against microglia-dependent amyloid-beta toxicity through inhibiting NF-Kappa b signaling (5).

References:

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2. Chen, W. Y. *et al.* (2005) Cell **123**:437.
3. Huffmun, D. M. *et al.* (2007) Cancer Res. **67**:6612.
4. Mattagajasingh, I. *et al.* (2007) Proc. Natl. Acad. Sci. **104**:14855.
5. Chen, J. *et al.* (2005) J. Biol. Chem. **280**:40364.

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