Recombinant Human Sirtuin 5/SIRT5 His-tag

Catalog Number: 10270-DA

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived human Sirtuin 5/SIRT5 protein Met33-Ser310 Accession # Q9NXA8 with a C-terminal 6-His tag
N-terminal Sequence Analysis	Met33 & Ser37
Predicted Molecular Mass	31 kDa

SPECIFICATIONS

SDS-PAGE	33 kDa under reducing conditions
Activity	Measured by its ability to remove the succinyl group from a fluorogenic peptide substrate in a coupled assay. The specific activity is >20 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, 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 and Brij-35. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM Tris, 150 mM NaCl, 1 mM DTT, pH 8.0
- Stop Solution: 8 ng/µL Recombinant Human Active Trypsin 3/PRSS3 (Catalog # 3714-SE), 4 mM Nicotinamide (Sigma, Catalog # 72340), 50 mM Tris, 100 mM NaCl, 30% (v/v) isopropanol, pH 8.0
- Recombinant Human Sirtuin 5/SIRT5 (rhSIRT5) (Catalog # 10270-DA)
- Nicotinamide adenine dinucleotide sodium salt (β-NAD) (Sigma, Catalog # N6522), 100 mM stock in diH2O
- Substrate: FLUOR DE LYS®-Succinyl, Desuccinylase Substrate (5 mM) (Enzo Life Sciences Int., Catalog # BML-KI590)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhSIRT5 to 1 ng/µL in Assay Buffer.
2. Dilute Substrate to 100 µM in Assay Buffer containing 2 mM β-NAD.
3. Combine 25 µL of 1 ng/µL rhSIRT5 and 25 µL of dilute Substrate in plate. For Control, load 25 µL of 1 ng/µL rhSIRT5 alone.
4. Seal plate and incubate at 37 °C for 30 minutes.
5. Add 50 µL of Stop Solution to all wells and mix. For Control well(s), add 25 µL of dilute Substrate after addition of Stop Solution.
6. Seal plate and incubate at room temperature for 15 minutes.
7. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in endpoint mode.
8. Calculate specific activity:

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

*Adjusted for Control
**Derived using calibration standard 7-amino, 4-Methyl Coumarin (AMC) (Sigma, Catalog # A9891).

Final Assay Conditions

Per Well:

- rhSIRT5: 0.025 µg
- Substrate: 25 µM
- β-NAD: 0.5 mM

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, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

DATA

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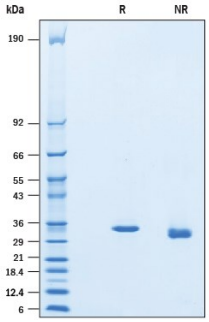
SDS-PAGE



2 µg/lane of Recombinant Human Sirtuin 5/SIRT5 His-tag was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing a band at 33 kDa under reducing conditions.

BACKGROUND

Sirtuin 5, encoded by the SIRT5 gene, is a nicotinamide adenine dinucleotide (NAD)-dependent enzyme that is part of the SIRT family also known as the SIR2 (silent information regulator 2)-like protein family. The SIR2 family of enzymes is classified as class III histone deacetylases (HDACs). The family is comprised of seven family members, SIRT1-7 that contain the conserved NAD-binding and catalytic domains but have distinct N- and C-termini that contribute differences in specificity, subcellular localization and enzymatic activity (1,2). SIRT5 is a 310 amino acid protein with broad tissue distribution (3,4) and primarily localized to the mitochondrial matrix (3,4) with a larger acyl binding pocket and unique active site residues compared to other SIRTs (1,2). SIRT5 possesses very weak deacetylase activity but can perform protein desuccinylation, demalonylation, and deglutarylation (1,2) due to its altered binding pocket. It is the principal regulator of these modifications in mitochondrial as well as the cytosolic and nuclear proteins based on proteomic detection of hundreds of SIRT5 substrate molecules (5-9). SIRT5 is implicated to impact cellular processes including metabolism (9-11) and fatty acid B-oxidation (6,11,12) in maintenance of cardiac homeostasis (12,13), cancer (14-16), and neurodegenerative disorders (17,18). Given its role in many pathologies, there is significant interest in targeting SIRT5 therapeutically (11,16).

References:

1. Peng, C. *et al.* (2011) Mol. Cell Proteomics **10**:M111.012658.
2. Du, J. *et al.* (2011) Science. **334**:806.
3. Michishita, E. *et al.* (2005) Mol. Biol. Cell **16**:4623.
4. Nakagawa, T. *et al.* (2009) Cell **137**:560.
5. Park, J. *et al.* (2013) Mol. Cell **50**:919.
6. Rardin, M.J. *et al.* (2013) Cell Metab. **18**:920.
7. Tan, M. *et al.* (2014) Cell Metab. **19**:605.
8. Colak, G. *et al.* (2015) Mol. Cell Proteomics **14**:3056.
9. Nishida, Y. *et al.* (2015) Mol. Cell **59**:321.
10. Wang, F. *et al.* (2017) Cell Rep. **19**:2331.
11. Kumar, S and D. B. Lombard. (2018) Crit. Rev. Biochem. Mol. Biol. **53**:311.
12. Sadhukhan, S. *et al.* (2016) Proc. Natl. Acad. Sci. USA **113**:4320.
13. Hershberger, K.A. *et al.* (2017) J. Biol. Chem. **292**:19767.
14. Lu, W. *et al.* (2014) Tumour Biol. **35**:10699.
15. Zhao, T. *et al.* (2017) Oncotarget **8**:53819.
16. Bringman-Rodenbarger, L.R. *et al.* (2018) Antioxid. Redox Signal **28**:677.
17. Li, F and L. Liu. (2016) Front. Cell Neurosci. **10**:171.
18. Koronowski, K.B. *et al.* (2018) Front. Neurosci. **12**:32.

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