Recombinant Human Sirtuin 2/SIRT2

Catalog Number: 4358-DA

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived Met1-Gln389, with a C-terminal 6-His tag Accession # Q8IXJ6
N-terminal Sequence Analysis	No results obtained: Presumed blocked
Predicted Molecular Mass	44 kDa

SPECIFICATIONS

SDS-PAGE	43-49 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 >3 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	>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 and Glycerol. 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 2/SIRT2 (rhSIRT2) (Catalog # 4358-DA)
- β-Nicotinamide adenine dinucleotide hydrate (β-NAD) (Sigma, Catalog # N6522), 100 mM stock in diH_2O
- Substrate: Ac-Arg-Gly-Lys(Ac)-AMC (Catalog # ES016), 20 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhSIRT2 to 0.04 µg/µL in Assay Buffer.
2. Dilute Substrate to 500 µM in Assay Buffer containing 2 mM β-NAD.
3. Mix 25 µLof 0.04 µg/µL rhSIRT2 and 25 µL of Substrate in plate. Add 25 µL of 0.04 µg/µL rhSIRT2 alone for the control.
4. Cover with parafilm or a plate sealer and incubate at 37 °C for 30 minutes.
5. Add 50 µL of Stop Solution to all wells. Mix. For control well(s), add 25 µL of Substrate after addition of Stop Solution.
6. Seal tightly 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 # A-9891).

Final Assay Conditions

Per Well:

- rhSIRT2: 1 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, -20 to -70 °C as supplied. ● 3 months, -20 to -70 °C under sterile conditions after opening.

BACKGROUND

Sirtuin 2, encoded by the SIRT2 gene, is also known as SIR2 (silent information regulator 2)-like protein 2. It is a nicotinamide adenine dinucleotide (NAD)-dependent histone/protein deacetylase (1, 2). The SIR2 family of enzymes is classified as class III histone deacetylases (HDACs) and has been implicated in many cellular processes that include histone deacetylation, gene silencing, chromosomal stability, and aging (3, 4). Unlike class I and class II HDACs, the enzymatic activity of class III HDACs is NAD dependent and insensitive to HDAC inhibitor trichostatin A (5).

References:

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3. North, B.J. and Verdin, E. (2004) Genome Biol. **5**:224.
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5. Gray, S.G. and Ekstrom, T.J. (2001) Exp. Cell Res. **262**:75.

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