



## Sirtuin 1 (193-741). E.coli Active Enzyme HDAC Type 3,-1; Silent mating type information regulation 2 homolog 1

### BACKGROUND

Human Sirtuin 1 (GenBank Accession No. NM\_012238), amino acids 193-741 with N-terminal GST tag, MW=87.2 kDa, expressed in E.coli expression system.

Sirtuin 1 is a NAD-dependent deacetylase, which regulates processes such as apoptosis and muscle differentiation by deacetylating key proteins. Deacetylates Lys-382 of p53/TP53 and impairs its ability to induce proapoptotic program and modulate cell senescence. Deacetylates TAF1B and thereby represses rDNA transcription by the RNA polymerase I. Involved in HES1- and HEY2-mediated transcriptional repression. Inhibits skeletal muscle differentiation by deacetylating PCAF and MYOD1. May serve as a sensor of the cytosolic ratio of NAD(+)/NADH, which is essential in skeletal muscle cell differentiation. Despite some ability to deacetylate histones in vitro, such activity is either weak or nonexistent in vivo. Inhibited by nicotinamide. Activated by resveratrol (3,5,4'-trihydroxy-trans-stilbene), butein (3,4,2',4'- tetrahydroxychalcone), piceatannol (3,5,3',4'-tetrahydroxy-trans-stilbene), Isoliquiritigenin(4,2',4'-trihydroxychalcone), fisetin (3,7,3',4'-tetrahydroxyflavone) and quercetin (3,5,7,3',4'- pentahydroxyflavone).<sup>3</sup>

### ACTIVITY

Useful for the study of enzyme kinetics, screening inhibitors, and selectivity profiling. 10.2 pmol/min/ $\mu$ g. One U =1 pmol/min, Assay condition: 25 mM Tris/HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl<sub>2</sub>, and 0.1 mg/ml BSA, 20  $\mu$ M HDAC substrate 1, 500  $\mu$ M NAD<sup>+</sup> and Sirtuin 1. Incubation condition: 30 min at 37°C followed by treatment with 50  $\mu$ l of HDAC developer for 15 min at RT.

### PURITY

> 60%

### APPLICATIONS

Enzyme activity assay standard

### ORDERING INFORMATION

**CATALOG NUMBER**  
X2037E

**SIZE**  
100  $\mu$ g

**CUSTOMER STORAGE**  
Product should be stored at -80°C.  
Aliquot to avoid freeze/thaw cycles

**FORMULATION**  
Provided in 50 mM Tris-HCl, pH 8.0,  
138 mM NaCl, 20 mM glutathione, and  
10% glycerol.

**SHIP CONDITIONS**  
Ship on dry ice, freeze upon arrival

**STABILITY**  
Products are stable for one year from  
purchase when stored properly

**CONCENTRATION**  
Lot Specific

**SOURCE**

## **ASSAY METHODS**

### **MATERIALS**

1. 4 ul of Sirtuin 1 (0.25 ug/ul)
2. 31 ul of HDAC assay buffer (Exalpha catalog number xxxx)
3. 5 ul of 1 mg/ml BSA
4. 5 ul of 2 mM NAD+
5. 5 ul of 300 uM substrate (Biomol catalog number: KI-177 gave the best results)

### **PROCEDURE**

1. Add all reaction mixture to a low binding black plate
2. Incubate at 30°C for 60 minutes
3. Stop the reaction by adding 50 ul of HDAC assay developer and incubate the plate at rt for 15 minutes.
4. Read sample in microplate reader at a wavelength of 350-380 nm and detection of emitted light at 440-460nm.

### **REFERENCES**

1. Kolthur-Seetharam, U. et al (2006) Cell Cycle 5, 873-877.
2. Solomon, J.M. et al(2006) Mol. Cell. Biol. 26, 28-38.
3. UCSC Genome Browser-<http://genome.ucsc.edu/>, Specific for Sirtuin 1:  
[http://genome.ucsc.edu/cgi-bin/hgGen?db=hg18&hgg\\_gene=NM\\_012238&hgg\\_chrom=chr10&hgg\\_start=69314432&hgg\\_end=69348147](http://genome.ucsc.edu/cgi-bin/hgGen?db=hg18&hgg_gene=NM_012238&hgg_chrom=chr10&hgg_start=69314432&hgg_end=69348147), Accessed March 13, 2007.

### **PRODUCT SPECIFIC REFERENCES**