PRODUCT DATA SHEET

SIRT2 (SIR2L, SIR2L2)

CATALOG NO.: KDA-11-178 **LOT NO.:**

DESCRIPTION: Human recombinant SIRT2 (residues 50-389 (end); Genbank Accession # NM_012237; MW = 36.5 kDa) expressed in *E. coli* with an C-terminal His-tag. Catalyzes the deacetylation of protein acetyllysine residues in a reaction that forms nicotinamide and *O*-acetyl-ADP-ribose from the co-substrate NAD^+ and the lysine's acetyl function. In contrast to SIRT1, which is the closest human homolog to yeast Sir2, SIRT2[1] is the ortholog of another yeast sirtuin, Hst2[2,3], which like Sir2, but independently of it, mediates yeast replicative lifespan extension by calorie restriction[4]. While SIRT2 is primarily located in the cytoplasm, where it can form a complex with HDAC6 and function as an α-tubulin deacetylase[5], it can also undergo cell cycle-linked nucleo-cytoplasmic shuttling[6-9] and deacetylate various histone and non-histone, cytoplasmic and nuclear substrates (see reviews[10,11]). SIRT2's connections to mitotic regulatory processes include its phosphorylation by Cdk1/cyclin B1, its dephosphorylation by CDC14A/B[12] and its enhancement, via deacetylation of both SET8 (PR-Set7) and histone H4K16, of SET8 monomethylation of histone H4K20[9], an essential event in cell cycle progression. Depending on the particular cancer type and context, it appears that SIRT2 may act as either a tumor suppressor[13,14] or tumor promoter[15,16] (see also review[11]). There is evidence that SIRT2 inhibition may be beneficial for neurodegenerative disorders, including Parkinson's disease[17] and Huntington's disease[18] (but see[19]), possibly by decreasing sterol biosynthesis via inhibition of SREBP-2 translocation to the nucleus[18] and/or effects on microtubule-mediated translocation of neurotoxic proteins such as oligomeric α-synuclein[17] (see also review[10]).

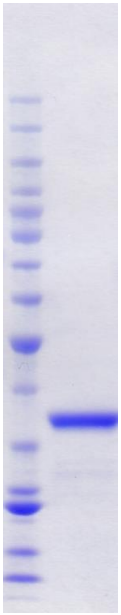
PURITY: >90% by SDS-PAGE

ASSAY CONDITIONS: RBC's SIRT2 displays NAD^+-dependent deacetylase activity in an endpoint, trypsin-coupled reaction with a fluorogenic substrate. The deacetylation reaction is performed in 50 mM Tris-HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 1 mg/ml BSA, with Ac-RHK-K(Ac)-AMC and NAD^+ as substrates (K_m's are ~200 and 500 µM respectively). The reaction is terminated and fluorescence signal (Ex. 360 nm/Em. 460 nm) developed (~30 min.) by addition of an equal volume of 2 mM nicotinamide, 16 mg/mL trypsin in 50 mM Tris-HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$.

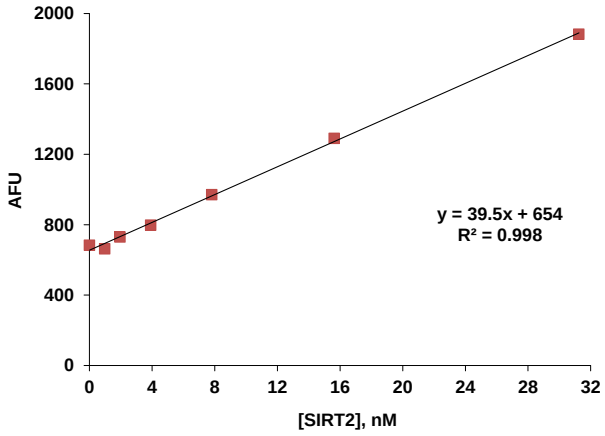
SUPPLIED AS: __ µg/µl total protein in 50 mM Tris/HCl pH 7.5, 500 mM NaCl, 1 mM TCEP, 10% glycerol (v/v) as determined by OD_{280}

STORAGE: -70°C. Thaw quickly and store on ice before use. The remaining, unused, undiluted enzyme should be snap frozen, for example in a dry/ice ethanol bath or liquid nitrogen. Minimize freeze/thaws if possible, but very low volume aliquots (<5 µl) or storage of diluted enzyme is not recommended.

REFERENCES: 1) R.A. Frye *Biochem. Biophys. Res. Commun.* 1999 **260** 273; 2) R.A. Frye *Biochem. Biophys. Res. Commun.* 2000 **273** 793; 3) S. Perrod *et al. EMBO J.* 2001 **20** 197; 4) D.W. Lamming *et al. Science* 2005 **309** 1861; 5) B.J. North *et al. Mol. Cell* 2003 **11** 437; 6) S.C. Dryden *et al. Mol. Cell. Biol.* 2003 **23** 3173; 7) A. Vaquero *et al. Genes Dev.* 2006 **20** 1256; 8) B.J. North & E. Verdin *PLoS One* 2007 **2** e784; 9) L. Serrano *et al. Genes Dev.* 2013 **27** 639; 10) G. Donmez & T.F. Outeiro *EMBO Mol. Med.* 2013 **5** 344; 11) Y.I. Cha & H. Kim *BMB Rep.* 2013 **46** 429; 12) B.J. North & E. Verdin *J. Biol. Chem.* 2007 **282** 19546; 13) M. Hiratsuka *et al. Biochem. Biophys. Res. Commun.* 2003 **309** 558; 14) C.C. Lai *et al. Tumour Biol.* 2013 **34** 1847; 15) P.Y. Liu *et al. Cell Death Differ.* 2013 **20** 503; 16) M.H. Yang *et al. Mol. Cancer Res.* 2013 **11** 1072; 17) T.F. Outeiro *et al. Science* 2007 **317** 516; 18) R. Luthi-Carter *et al. Proc. Natl. Acad. Sci. USA* 2010 **107** 7927; 19) A. Bobrowska *et al. PLoS One* 2012 **7** e34805



Coomassie blue stained SDS-PAGE (4-12% acrylamide) of 4 µg of purified SIRT2. MW markers at left are from the top: 220, 160, 120, 100, 90, 80, 70, 60, **50**, 40, 30, 25, **20**, 15 & 10 kDa.



Assay of SIRT2 Lysine Deacetylase Activity. Reactions were 30 min., 37°C with 200 µM Ac-RHK-K(Ac)-AMC and 500 µM NAD^+ as substrates. Fifty µL reactions were performed in a white 96-well plate (Corning 3992) and fluorescence read, after development, in a Fluoroskan Ascent FL fluorimeter (Thermo). Slope of the plot (39.5 AFU/nM/30 min.) corresponds to a turnover number of 2.50 min^{-1} or a specific activity of 68.2 pmol/min./µg under these conditions. (Calculated from an AMC standard curve, slope = 526 AFU/µM.)

This product is NOT intended for therapeutic or diagnostic use in animals or in humans.

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