PRODUCT DATA SHEET

SIRT1 (Sir2-like protein 1)

CATALOG NO.: KDA-11-174 **LOT NO.:**

DESCRIPTION: Human recombinant SIRT1 (residues 1-747 (end); Genbank Accession # NM_012238; MW = 83.2 kDa) expressed in *E. coli* with an N-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[1-3]. SIRT1 is the closest human homolog[4] of yeast Sir2 (Silent information regulator 2), the founding exemplar of the '"sirtuins" or class III (NAD^+-dependent) histone deacetylases (HDACs). The wide array of *in vivo* targets for SIRT1 deacetylation[5] includes other proteins involved in regulatory post-translational modifications[6,7], several histone sites (H4K16, H3K9/K14, H1K26)[1,8], multiple transcription factors (e.g. p53[9], NF-κB[10], FOXOs[11], PGC-1α[12]) and key metabolic enzymes[13, 14]. SIRT1 has regulatory roles in gene expression[15] and gene silencing[7], in cellular stress responses (e.g to DNA damage[16], oxidation[17] and heat[18]), in autophagy[19], in apoptosis and cell survival[9, 20, 21] and in the circadian clock[22, 23]. SIRT1 activity is upregulated in response to calorie restriction (CR), in part by increased expression[24] in part by the elevation of cellular NAD^+[25], and is required for downstream CR effects[26-28]. Given the benefits of CR for the diseases of aging (and possibly longevity *per se*)[30], the discovery of natural (e.g. resveratrol)[31] and synthetic[32,33] small molecule SIRT1 activators opened a promising avenue for therapeutic interventions in, for example, type 2 diabetes[33] and neurodegeneration[34]. While the question of whether these compounds act by directly stimulating SIRT1 catalytic activity has been controversial for some years[35-37], recent results indicate that this is most likely in fact the case[38] and that activators bind an allosteric site on SIRT1[39].

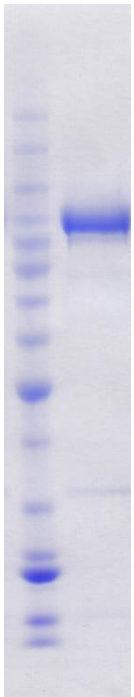
PURITY: >85% by SDS-PAGE

ASSAY CONDITIONS: RBC's SIRT1 displays NAD^+-dependent deacetylase activity in an endpoint, trypsin-coupled reaction with a fluorogenic substrate, Ac-RHK-K(Ac)-AMC, based on residues 379-382 of p53. 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 substrates as indicated (see Figure below). 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 $MgCl_2$.

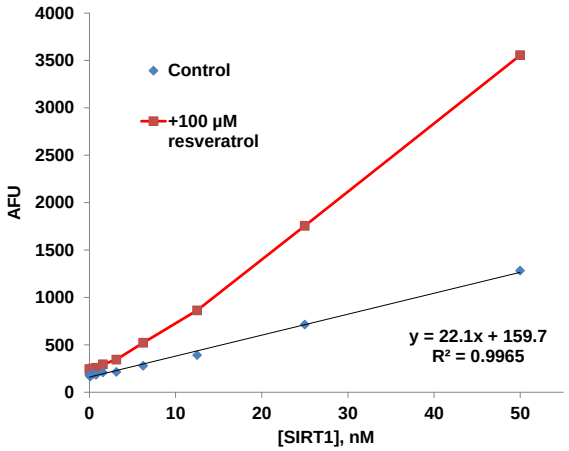
SUPPLIED AS: ___ µg/µl total protein in 50 mM Tris/HCl pH 7.5, 150 mM NaCl, 10% glycerol (v/v) as determined by OD_{280}.

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) S Imai *et al. Nature* 2000 **403** 795; 2) K.G. Tanner *et al. Proc. Natl. Acad. Sci. USA* 2000 **97** 14178; 3) J.C. Tanny and D. Moazed *Proc. Natl. Acad. Sci. USA* 2000 **98** 415; 4) R. A. Frye *Biochem. Biophys. Res. Commun.* 2000 **273** 793; 5) Y. Chen *et al. Mol. Cell. Proteomics* 2012 **11** 1048; 6) T. Bouras *et al. J. Biol. Chem.* 2005 **280** 10264; 7) A. Vaquero *et al. Nature* 2007 **450** 440; 8) A. Vaquero *et al. Mol. Cell* 2004 **16** 93; 9) H. Vaziri *et al. Cell* 2001 **107** 149; 10) F. Yeung *et al. EMBO J.* 2004 **23** 2369; 11) A. Brunet *et al. Science* 2004 **303** 2011; 12) S. Nemoto *et al. J. Biol. Chem.* 2005 **280** 16456; 13) W.C. Hallows *et al. Proc. Natl. Acad. Sci. USA* 2006 **103** 10230; 14) W.C. Hallows *et al. J. Biol. Chem.* 2012 **287** 3850; 15) T. Zhang & W.L. Kraus *Biochim. Biophys. Acta* 2010 **1804** 1666; 16) M. Gorospe & R. de Cabo *Trends Cell Biol.* 2008 **18** 77; 17) Y. Kawai *et al.J. Biol. Chem.* 2011 **286** 7629; 18) S.D. Westerheide *et al. Science* 2009 **323** 1063; 19) I.H. Lee *et al. Proc. Natl. Acad. Sci. USA* 2008 **105** 3374; 20) J. Luo *et al. Cell* 2001 **107** 137; 21) Y.S. Hori *et al. PLoS One* 2013 **8** e73875; 22) G. Asher *et al. Cell* 2008 **134** 317; 23) Y. Nakahata *et al. Cell* 2008 **134** 329; 24) H.Y. Cohen *et al. Science* 2004 **305** 390; 25) C. Canto *et al. Nature* 2009 **458** 1056; 26) G. Boily *et al. PLoS One* 2008 **3** e1759; 27) D. Chen *et al. Science* 2005 **310** 1641; 28) D.E. Cohen *et al. Genes Dev.* 2009 **23** 2812; 30) L. Guarente *Genes Dev.* 2013 **27** 2072; 31) K.T. Howitz *et al. Nature* 2003 **425** 191; 32) H. Yang *et al. Aging Cell* 2007 **6** 35; 33) J.C. Milne *et al. Nature* 2007 **450** 712; 34) D. Kim *et al. EMBO J.* 2007 **26** 3169; 35) M. Kaeberlein *et al. J. Biol. Chem.* 2005 **280** 17038; 36) M.T. Borra *et al. J. Biol. Chem.* 2005 **280** 17187; 37) M. Pacholec *et al. J. Biol. Chem.* 2010 **285** 8340; 38) H. Dai *et al. J. Biol. Chem.* 2010 **285** 32695; 39) B.P. Hubbard *et al. Science* 2013 **339** 1216



Coomassie blue stained SDS-PAGE (4-12% acrylamide) of 4 µg of purified SIRT1. MW markers (left) are, from top, 220, 160, 120, 100, 90, 80, 70, 60, **50**, 40, 30, 25, **20**, 15, 10 kDa. Note that SIRT1 migrates at an anomalously high position relative to its actual mass, 83.2 kDa.



SIRT1 Assayed in the Presence or Absence of Resveratrol. Deacetylation reactions were 30 min., 37°C with 25 µM Ac-RHK-K(Ac)-AMC and 100 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). The slope of the control plot (22.1 AFU/nM/30 min.) corresponds to a turnover number of 68.8 h^{-1} or a specific activity of 13.8 pmol/min./µg under these conditions. (Calculated from an AMC standard curve, slope = 642 AFU/µM.)

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

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