PRODUCT DATASHEET

HDAC6 (Histone deacetylase 6; HD6)

CATALOG NO.: KDA-21-213 **LOT NO.:**

DESCRIPTION: Full-length human recombinant HDAC6 (residues 1-1215 (C-terminus); Genbank Accession # NM_006044; MW = 158.3 kDa) expressed in *Sf9* insect cells with an N-terminal GST-tag. Catalyzes the deacetylation of protein acetyllysine residues. HDAC6, along with HDAC10, is classified as a class IIb HDAC. Class II HDACs, like class I (HDACs 1-3, 8) and class IV (HDAC11) enzymes are Zn(II)-dependent amidohydrolases, mechanistically and phylogenetically distinct from the NAD^+-dependent sirtuin deacetylases (class III HDACs). HDAC6 is unique in that it comprises two full HDAC catalytic domains[1,2]. Primarily a cytoplasmic enzyme[3], HDAC6 can form a complex with SIRT2[4] and act as a tubulin deacetylase[3,5,6]. Aside from its HDAC domains, HDAC6 contains a dynein interaction domain[7] and a zinc-finger domain that binds the unconjugated C-termini of ubiquitin found in protein aggregates[8]. HDAC6 functions in multiple stress-response pathways for alleviating the effects of misfolded and aggregated proteins, including aggresome formation[7,8], autophagy[9,10], the positive regulation of Hsp90 chaperone activity[11-13] and the activation of HSF1 and consequent increased expression of additional chaperones[14]. HDAC6 activity can promote oncogenesis[15] and selective HDAC6 inhibition[16], perhaps especially in combination with DNA damaging agents[17], is considered a promising approach for anti-cancer therapy. Evidence for cigarette smoke-induced and HDAC6-dependent autophagic shortening of cilia in airway epithelia has led to the suggestion of HDAC6 inhibition as a possible therapeutic approach for COPD[18]. There is evidence that HDAC6 inhibition might be beneficial for some neurodegenerative disorders[19] including Alzheimer's disease[20]. However, in other neurodegenerative disease model systems, HDAC6 overexpression confers benefits[21], as might be expected from its role in promoting the elimination of protein aggregates[10, 14].

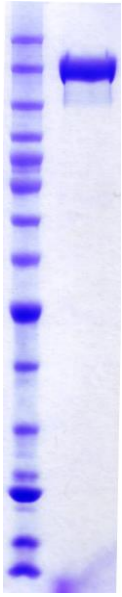
PURITY: >95% by SDS-PAGE

ASSAY CONDITIONS: RBC's HDAC6 displays lysine 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 RHK-K(Ac)-AMC as substrate (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 µM Trichostatin A, 16 mg/mL trypsin in 50 mM Tris-HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$.

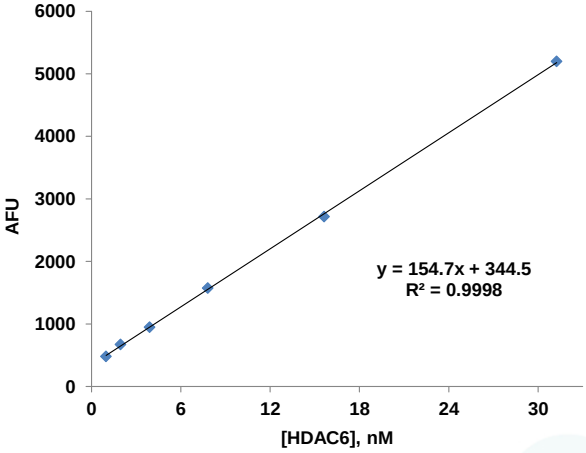
SUPPLIED AS: __ µg/µl total protein in 50 mM Tris/HCl pH 7.5, 500 mM NaCl, 10% glycerol (v/v) as determined by OD_{280}.

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) I.V. Gregoretti *et al. J Mol Biol* 2004 **338** 17; 2) Y. Zhang *et al. J. Biol. Chem.* 2006 **281** 2401; 3) C. Hubbert *et al. Nature* 2002 **417** 455; 4) B.J. North *et al. Mol. Cell* 2003 **11** 437; 5) A. Matsuyama *et al. EMBO J* 2002 **21** 6820; 6) Y. Zhang *et al. EMBO J* 2003 **22** 1168; 7) Y. Kawaguchi *et al. Cell* 2003 **115** 727; 8) H. Ouyang *et al. J. Biol. Chem.* 2012 **287** 2317; 9) A. Iwata *et al. J. Biol. Chem.* 2005 **280** 40282; 10) U.B. Pandey *et al. Nature* 2007 **447** 859; 11) J.J. Kovacs *et al. Mol. Cell* 2005 **18** 601; 12) P. Bali *et al. J. Biol. Chem.* 2005 **280** 26729; 13) P.J Murphy *et al. J. Biol. Chem.* 2005 **280** 33792; 14) C. Boyault *et al. Genes Dev.* 2007 **21** 2172; 15) Y.S. Lee *et al. Cancer Res.* 2008 **68** 7561; 16) P. Yang *et al. Drug Discov. Ther.* 2013 7 233; 17) J.H. Lee *et al. Proc. Natl. Acad. Sci. USA* 2013 **110** 15704; 18) H.C. Lam *et al. J. Clin. Invest.* 2013 **123** 5212; 19) M.A. Rivieccio *et al. Proc. Natl. Acad. Sci. USA* 2009 **106** 19599; 20) N. Govindarajan *et al. EMBO Mol. Med.* 2013 **5** 52; 21) G. Du *et al. Mol. Biol. Cell* 2010 **21** 2128



Coomassie blue stained SDS-PAGE (4-12% acrylamide) of 4 µg of purified HDAC6. 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 HDAC6 Lysine Deacetylase Activity. Reactions were 90 min., 37°C with 50 µM RHK-K(Ac)-AMC as substrate. 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 (154.7 AFU/nM/90 min.) corresponds to a turnover number of 3.13 min^{-1} or a specific activity of 19.8 pmol/min./µg under these conditions. (Calculated from an AMC standard curve, slope = 550 AFU/µM.)

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