REACTION BIOLOGY

PRODUCT DATASHEET

HDAC 4 (Histone deacetylase 4)

CATALOG NO.: KDA-21-279 **LOT NO.:**

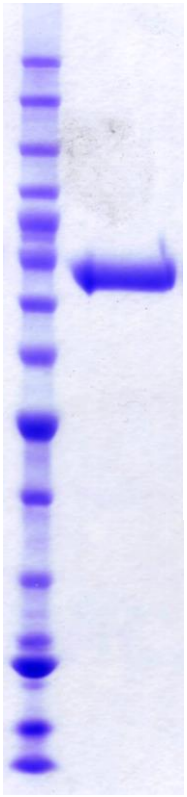
DESCRIPTION: Human recombinant HDAC4 (residues 627-1084; Genbank Accession # NM_006037; MW = 76.9 kDa) expressed in insect cells with both a N-terminal GST-fusion tag and a C-terminal His tag.

PURITY: >95% by SDS-PAGE

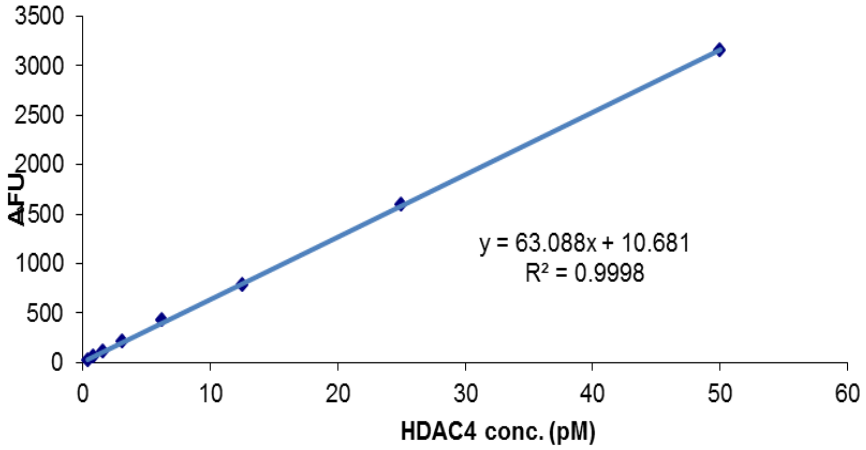
ASSAY CONDITIONS: RBC's HDAC4 displays lysine deacetylase activity in an endpoint, trypsin-coupled reaction with a fluorogenic substrate. The deacetylation reaction is performed in 50 mM Tris-HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 1 mg/ml BSA, with Boc-K(Ac)-AMC as substrate (see Figure 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 uM 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 in 50 mM Tris HCl, pH 7.5, 500 mM NaCl, 10% glycerol as determined by OD_{280}.

STORAGE: -70°C. Thaw quickly and store on ice before use. The remaining, unused, undiluted protein 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.



Coomassie blue-stained SDS-PAGE (4-12% acrylamide) of 2 µg of RBC HDAC4. MW markers (left) are, from top, 220, 160, 120, 100, 90, 80, 70, 60, **50**, 40, 30, 25, **20**, 15, 10 kDa.



Assay of HDAC4 Lysine Deacetylase Activity. Reactions were 60 min., 37°C with 100 µM Boc-K(TFA)-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 (63.088 AFU/pM/60 min.) corresponds to a specific activity of 25 nmol/min./µg under these conditions. (Calculated from an AMC standard curve, slope = 550 AFU/µM.)

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