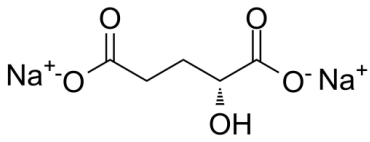
Data Sheet

Product Name:	D-alpha-Hydroxyglutaric acid (disodium salt)
Cat. No.:	CS-6030
CAS No.:	103404-90-6
Molecular Formula:	C5H6Na2O5
Molecular Weight:	192.08
Target:	Reactive Oxygen Species
Pathway:	Immunology/Inflammation; Metabolic Enzyme/Protease; NF-κB
Solubility:	H2O : ≥ 32 mg/mL (166.60 mM)



BIOLOGICAL ACTIVITY:

D-alpha-Hydroxyglutaric acid disodium salt is a weak competitive α-Ketoglutarate(**α-KG**)-dependent dioxygenase inhibitor with **K_i** of 10.87±1.85 mM. K_i for L-Hydroxyglutaric acid (L-2-HG) is 0.628±0.036 mM. IC50 & Target: Ki: 10.87±1.85 mM (α-KG)[1] **In Vitro:** Addition of 50 mM and 100 mM of D-alpha-Hydroxyglutaric acid (D-2-HG) results in partial and nearly complete inhibition of CeKDM7A, respectively. To further examine the mode of interaction between α-KG and D-2-HG, CeKDM7A are incubated with a fixed concentration (50 mM) of D-2-HG and increasing amount of α-KG. A partial inhibition of KDM7A toward both H3K9me2 and H3K27me2 peptides is observed in the presence of 50 mM D-2-HG and 100 μM α-KG. Addition of 300 μM α-KG is capable of reversing the inhibition of CeKDM7A by 50 mM D-2-HG, indicating that D-2-HG is a weak competitive inhibitor against α-KG toward the CeKDM7A demethylase. D-2-HG with K_i of 10.87±1.85 mM for inhibiting KDM5B/JARID1B/PLU-1[1]. D-alpha-Hydroxyglutaric acid (R-2HG) increases the lifespan of C. elegans. (R)-2HG interacts distinctly with the α-KG dependent dioxygenases. The intracellular (R)-2HG levels are 20-100 fold higher in U87 and HCT 116 cells expressing IDH1(R132H) than in control cells. The elevated (R)-2HG levels are comparable to those found in cells treated with octyl (R)-2HG, and levels reported for IDH1-mutant tumor samples[2].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: [1]To assay human JHDM1A/KDM2A demethylase activity toward H3K36me2, His tagged JHDM1A is first obtained by transforming pET28a-JHDM1A into Escherichia coli BL21 and protein expression is induced by addition of 1 mM IPTG at 30°C when cell density reaches 0.5 OD_{600} units. Cells are lysed by sonication and Ni-NTA agarose is used to purify His-JHDM1A fusion proteins. Histone demethylase assay is carried out by incubating 2 μg oligonucleosomes, 4 μg purified His-JHDM1A, and/or 10-50 mM D-2-HG in histone demethylation buffer [50 mM HEPES (pH 8.0), 625 μM $Fe(NH_4)_2(SO_4)_2$, 0.1-0.5 mM α-KG, 2 mM ascorbate] at 37°C for 2-3 hr and the reactions are stopped by the addition of SDS loading buffer and subsequently analyzed by western blotting using anti-H3K36me2 antibody. To measure CeKDM7A demethylase activity toward H3K9me2 and H3K27me2, two synthetic dimethylated peptides H3K9me2 [ARTKQTARK (me2)STGGKA] and H3K27me2 [QLATKAARK (me2)SAPAS] are used as substrates. Demethylase assays are carried out in the presence of 10 μg enzyme, 1 μg peptide in 20 μl buffer 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 50 μM $(NH_4)_2Fe(SO_4)_2$, 100 μM α-KG, 2 mM Vc, 10 mM PMSF for 3 hr. The demethylation reaction mixture is desalted by passing through a C_{18} ZipTip. To examine the inhibitory effect of 2-HG, various concentrations of 2-HG are incubated with KDM7A briefly before adding other reaction mixtures. The samples are analyzed by a MALDI-TOF/TOF mass spectrometer[1]. **Cell Assay:** [2]U87 cells, HCT 116 IDH1(R132H/+) cells, and HEK 293 cells are seeded in 12-well plates and after overnight incubation are treated with indicated concentrations of each compound (e.g., 400 and 800 μM D-2-HG). After harvesting, cells are stained with Acridine Orange (AO) and DAPI. Cell number and viability are measured based on AO and DAPI fluorescence measured by NC3000[2].

References:

[1]. Xu W, et al. Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of α-ketoglutarate-dependent dioxygenases. Cancer Cell. 2011 Jan 18;19(1):17-30.

[2]. Fu X, et al. 2-Hydroxyglutarate Inhibits ATP Synthase and mTOR Signaling. Cell Metab. 2015 Sep 1;22(3):508-15.

CAIndexNames:

Pentanedioic acid, 2-hydroxy-, disodium salt, (2R)-

SMILES:

O=C([O-])[C@H](O)CCC([O-])=O.[Na+].[Na+]

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