Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-OTUD6A

Enzyme description:- His-OTUD6A 1-288 (full length)

Clone number:- SC20889

Source:- BL21 Recombinant

Tag:- N-terminal His_6-tag

Purification method:- Ni^{++}-Sepharose

Expression level:- 5 mg/L

Calculated molecular mass:-

Monoisotopic	35722 Da
Average Mass	35743 Da

[cycsteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.79

Purity:- 90%

Enzyme storage buffer:-

50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT

Storage temperature:- -80ºC

Assay:-

Ub-Rho110-Gly cleavage assay monitored by Ex/Em 485/535 nm

Assay buffer:-

40 mM Tris pH 7.5, 100 mM NaCl, 5 mM DTT, 0.01% Triton X-100, 0.005% Ovalbumin, 0.5 μM Ub-Rho110-Gly

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Clone Data Sheet

His-OTUD6A

Protein	His-OTUD6A 1-288 (full length)
Synonyms	DUBA-2
Clone Number	SC20889
Species	Human
Accession Number	Protein: Q7L8S5 DNA: NM_207320
Tags	N-terminal His_6 tag
Amino acid sequence of expressed protein	MGSSHHHHHHSSGLEVLFQGPGS**MDDPKSEQQRILRRHQRERQELQAQIRSLK NSVPKTDKTKRKQLLQDVARMEAEMAQKHRQELEKFQDDSSIESVVEDLAKMN LENRPPRSSKAHRKRERMESEERERQESIFQAEMSEHLAGFKREEEEKLAAIL GARGLEMKAIPADGHCMYRAIQDQLVFSVSVEMLRCRTASYMKKHVDEFLPFF SNPETSDSFGYDDFMIYCDNIVRTTAWGGQLELRALSHVLKTPIEVIQADSPT LIIGEEYVKKPIILVYLRYAYSLGEHYNSVTPLEAGAAGGVLPRLL**
Native sequence	in bold
Protease cleavage	Precission site underlined
Cloning sites	BamH1 / Not1

DNA sequence of insert

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GGATCCATGGATGATCCGAAGAGTGAACAGCAGCGCATACTGCGCCGCCACCA
ACGCGAGAGGCAGGAGCTGCAGGCCCAGATCCGGAGCTTAAAAAACTCGGTCC
CCAAGACCGACAAGACGAAAAGAAAGCAGTTGCTCCAAGACGTGGCCCGCATG
GAGGCCGAGATGGCTCAGAAGCACCGGCAGGAGCTGGAGAAGTTCCAAGACGA
CAGTAGCATTGAATCTGTCGTCGAAGACCTGGCCAAGATGAATCTGGAAAACC
GGCCTCCCCGCTCCTCCAAAGCCCACAGAAAGAGAGAAAGAATGGAGTCCGAG
GAGAGGGAGCGCCAGGAGAGCATCTTCCAGGCTGAGATGTCGGAGCACCTGGC
CGGCTTCAAGCGCGAGGAGGAGGAGAAGCTCGCCGCCATCCTGGGAGCCAGGG
GTCTGGAGATGAAAGCGATCCCGGCCGACGGCCACTGCATGTACCGCGCCATC
CAAGACCAGCTGGTGTTCAGCGTGTCTGTGGAGATGCTGCGCTGCCGCACCGC
CAGCTACATGAAGAAGCACGTCGACGAGTTCCTGCCCTTCTTCAGCAACCCCG
AGACCAGCGACTCCTTCGGCTACGACGACTTCATGATCTACTGCGACAACATC
GTGCGCACCACGGCATGGGGAGGCCAGCTGGAGCTGAGGGCCCTGTCGCACGT
CCTGAAGACCCCCATCGAGGTGATCCAGGCCGACTCGCCCACCTTGATCATCG
GGGAGGAGTACGTCAAGAAGCCGATCATCCTGGTCTACCTGCGCTATGCCTAC
AGCCTCGGCGAGCACTACAACTCCGTGACACCGCTCGAGGCCGGCGCCGCCGG
GGGCGTGCTCCCGCGTCTCCTGTAGGCGGCCGC
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