Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of Dac-Ubiquitin

Enzyme description:- ubiquitin 1-76 = full length

Clone number:- DU26159

Source:- human Recombinant

Tag:- N-terminal Dac-

Purification method:- Ampicillin-Sepharose

Expression system:- E.coli

Calculated molecular mass:-

Monoisotopic	38154
Average Mass	38176

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.87

Purity:- 95%

Enzyme storage buffer:-

50 mM HEPES pH 7.5, 150mM NaCl, 1mM DTT

Storage temperature:- -80ºC

Assay:-

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Clone Data Sheet

Protein name Dac-ubiquitin (1-76)

Protein	Dac-Ubiquitin 1-76 = full length
Synonyms	
Clone Number	DU26159
Species	human
Accession Number	Protein: P62987
Tags	N-terminal Dac-
Aminoacid sequence of the expressed protein	MSAIPGVPQIDAESYILIDYNSGKVLAEQNADVRRDPASLTKMMTSY VIGQAMKAGKFKETDLVTIGNDAWATGNPVFKGSSLMFLKPGMQVPV SQLIRGINLQSGNDACVAMADFAAGSQDAFVGLMNSYVNALGLKNTH FQTVHGLDADGQYSSARDMALIGQALIRDVPNEYSIYKEKEFTFNGI RQLNRNGLLWDNSLNVDGIKTGHTDKAGYNLVASATEGQMRLISAVM GGRTFKGREAESKKLLTWGFRFFETVNPENLYFQGGSG**MQIFVKTLT GKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTL SDYNIQKESTLHLVLRLRGG**
Native sequence	ubiquitin (1-76) in bold. In mammalian cells Ubiquitin is expressed as a precursor by several genes and cleaved by a DUB to become the mature 76 residue protein Ubiquitin.
Protease cleavage	TEV protease underlined
Cloning sites	BamH1 / EcoR1
DNA sequence of the expression cassette	ATGTCTGCAATCCCGGGTGTACCGCAGATCGATGCGGAGTCCTACATCCTG ATTGACTATAACTCCGGCAAAGTGCTCGCCGAACAGAACGCAGATGTCCGC CGCGATCCTGCCAGCCTGACCAAAATGATGACCAGTTACGTTATCGGCCAG GCAATGAAAGCCGGTAAATTTAAAGAAACTGATTTAGTCACTATCGGCAAC GACGCATGGGCCACCGGTAACCCGGTGTTTAAAGGTTCTTCGCTGATGTTC CTCAAACCGGGCATGCAGGTTCCGGTTTCTCAGCTGATCCGCGGTATTAAC CTGCAATCGGGTAACGATGCTTGTGTCGCCATGGCCGATTTTGCCGCTGGT AGCCAGGACGCTTTTGTTGGCTTGATGAACAGCTACGTTAACGCACTGGGC CTGAAAAATACCCACTTCCAGACGGTACATGGTCTGGATGCTGATGGTCAG TACAGCTCCGCGCGAGATATGGCGCTGATCGGCCAGGCATTGATCCGTGAC GTACCGAATGAATACTCGATCTATAAAGAAAAAGAATTTACGTTTAACGGT ATTCGCCAGCTGAACCGTAACGGCCTGTTATGGGATAACAGCCTGAATGTC GACGGCATCAAAACCGGACACACTGACAAAGCAGGTTACAACCTTGTTGCT TCTGCGACTGAAGGCCAGATGCGCTTGATTTCTGCGGTAATGGGCGGACGT ACTTTTAAAGGCCGTGAAGCCGAAAGTAAAAAACTGCTAACCTGGGGCTTC CGTTTCTTTGAAACCGTTAACCCAGAAAACCTGTATTTTCAGGGCGGATCC GGTATGCAGATCTTCGTGAAGACCCTGACTGGTAAGACCATCACTCTCGAA GTGGAGCCGAGTGACACCATTGAGAATGTCAAGGCAAAGATCCAAGACAAG GAAGGCATCCCTCCTGACCAGCAGAGGTTGATCTTTGCTGGGAAACAGCTG GAAGATGGACGCACCCTGTCTGACTACAACATCCAGAAAGAGTCCACCCTG CACCTGGTCCTCCGTCTCcGAGGTGGGTGATAA