

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-UBE2D2

Enzyme description:- UBE2D2 2-147 (end)

Clone number:- DU4153

Source:- human recombinant

Tag:- N-terminal GST-tag

Purification method:- GSH-Sepharose

Expression level:- E.coli

Calculated molecular mass:-

Monoisotopic 43400 Da

Average Mass 43428 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.23

Purity:- 90%

Enzyme storage buffer:-

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

Storage temperature:- -80°C

Assay:-

Loading with Ubiquitin and UBE1 in the presence of Mg-ATP

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Clone Data Sheet

GST-UBE2D2

<u>Protein</u>	GST-UBE2D2 2-147 (end)
<u>Synonyms</u>	UbcH5b, E2D2
<u>Clone Number</u>	DU4153
<u>Species</u>	Human
<u>Accession Number</u>	Protein: P62837 DNA: NM_003339
<u>Tags</u>	N-terminal GST-tag
Aminoacid sequence of the expressed protein	MSPILGYWKIKGLVQPTRLLEYLEEKYEELHYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSR IAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLOGWQATFGGGDHPPKSD <u>LEVLFQGPLGSALKRIHKELNDLAR</u> DPPAQCSAGPVGDDMFHWQATIMGPNDSPYQGGVFFLTIHFPTDYPFKP PKVAFTRIYHPNINSNGSICLDILRSQWSPALTI SKVLLSICSLLCDP NPDDPLVPEIARIYKTDREKYNRIAREWTQKYAM
Native sequence	Start Methionine is missing
Protease cleavage	Prescission Protease site underlined
Cloning sites	BamH1 / NotI
<u>DNA sequence of insert</u>	<u>GGATCCGCTCTGAAGAGAATCCACAAGGAATTGAATGATCTGGCACGGGA</u> <u>CCCTCCAGCACAGTGTTTCAGCAGGTCTGTTGGAGATGATATGTTCCATT</u> <u>GGCAAGCTACAATAATGGGGCCAAATGACAGTCCCTATCAGGGTGGAGTA</u> <u>TTTTTCTTGACAATTCATTTCCCAACAGATTACCCCTTCAAACCACCTAA</u> <u>GGTTGCATTTACAACAAGAATTTATCATCCAAATATTAACAGTAATGGCA</u> <u>GCATTTGTCTTGATATTCTACGATCACAGTGGTCTCCAGCACTAACTATT</u> <u>TCAAAGTACTCTTGTCCATCTGTTCTCTGTTGTGTGATCCCAATCCAGA</u> <u>TGATCCTTTAGTGCCTGAGATTGCTCGGATCTACAAAACAGATAGAGAAA</u> <u>AGTACAACAGAATAGCTCGGGAATGGACTCAGAAGTATGCGATGTAAGCG</u> <u>GCCGC</u>