

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-UBE2E3

Enzyme description:- GST-UBE2E3 1-207 (full length)

Clone number:- DU14048

Source:- human recombinant

Tag:- N-terminal GST-tag

Purification method:- GSH-Sepharose

Expression system:- *E.coli*

Calculated molecular mass:-

Monoisotopic 49804 Da

Average Mass 49834 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.4

Purity:- 90%

Enzyme storage buffer:-

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

Storage temperature:- -80°C

Assay:-

Loading assay with SUMO and SAE1/SAE2 in the presence of Mg-ATP

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Clone Data Sheet

GST-UBE2E3

<u>Protein</u>	GST-UBE2E3 1-207 (full length)
<u>Synonyms</u>	UbcH9, E2E3, UbcM2
<u>Clone Number</u>	DU14048
<u>Species</u>	Human
<u>Accession Number</u>	Protein: NP_006348
<u>Tags</u>	N-terminal GST tag
Aminoacid sequence of the expressed protein	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGL EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVL DIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDHVTH PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIA WPLQGWQATFGGGDHPPKSD <u>LEVLFQGPLRS</u> MSSDRQRSDDESPSTSSGS SDADQRDPAAPEPEEQEERKPSATQOKKNTKLSSKTTAKLSTSAKRIQKE LAEITLDPPPNC SAGPKGDNIYWRSTILGPPGSVYEGGVFFLDITFSSD YPFKPPKVTFRTRIYHCNINSQGVICLDILKDNWSPALTI SKVLLSICSL LTDCNPADPLVGS IATQYLTNRAEHDRIARQWTKRYAT
Native sequence	in bold
Protease cleavage	Prescission protease site underlined
Cloning sites	BamH1 / NotI
<u>DNA sequence of insert</u>	AGATCTATGTCCAGTGATAGGCAAAGGTCCGATGATGAGAGCCCCAGCAC CAGCAGTGGCAGTTCAGATGCGGACCAGCGAGACCCAGCCGCTCCAGAGC CTGAAGAACAAGAGGAAAGAAAACCTTCTGCCACCCAGCAGAAGAAAAAC ACCAAACCTCTCTAGCAAAACCACTGCTAAGTTATCCACTAGTGCTAAAAG AATTCAGAAGGAGCTAGCTGAAATAACCCTTGATCCTCCTCCTAATTGCA GTGCTGGGCCTAAAGGAGATAACATTTATGAATGGAGATCAACTATACTT GGTCCACCGGGTCTGTATATGAAGGTGGTGTGTTTTTCTGGATATCAC ATTTTCATCAGATTATCCATTTAAGCCACCAAAGGTTACTTCCGCACCA GAATCTATCACTGCAACATCAACAGTCAGGGAGTCATCTGTCTGGACATC CTTAAAGACAACCTGGAGTCCCGCTTTGACTATTTCAAAGGTTTTGCTGTC TATTTGTTCCCTTTTGACAGACTGCAACCCTGCGGATCCTCTGGTTGGAA GCATAGCCACTCAGTATTTGACCAACAGAGCAGAACACGACAGGATAGCC AGACAGTGGACCAAGAGATACGCAACATAAGCGGCCGC