

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of Ubc H3 [2 - 236]

Enzyme description:- Ubc H3 [2 – 236]

Clone number:- DU 4164

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 0.5 mg/L

Calculated molecular mass:-

Monoisotopic 53, 395.63 daltons

Average Mass 53, 429.74 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 4.80

Purity:- 90 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay:- Ubiquitin assay

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Clone Data Sheet

Ubc H3 [2 - 236]

<u>Protein</u>	Ubc H3 [2 - 236]
<u>Clone Number</u>	DU 4164
<u>Species</u>	Human
<u>Accession number</u>	NM_004359
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSRIAYSKDFTLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFGGPLGSARPLVPSSQKALLL ELKGLQEEPVEGFRVTLVDEGDLYNWEVAIFGPPNTYYEGGYFKARLKF PIDYPYSPPAFRFLTKMWHPNYETGDVCISILHPPVDDPQSGELPSE WNPTQNVRTILLSVISLLNEPNTFSPANVDASVMYRKWKESKGDREYT DIIRKQVLGTVDAERDGVKVPTTLAEYCVKTKAPAPDEGSDLFYDDYY EDGEVEEEADSCFGDDEDDSGTEES
<u>Native sequence</u>	Amino acids A2 – S236 (end) of Ubc H3. Residue A232 of the fusion protein is equivalent to A2 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGPL</u>) residues 221 - 229
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I site of pGex6P-1

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Nucleotide Sequence

ATGTCCCCTATACTAGGTTATTGGAAAAATTAAGGGCCTTGTGCAACCCA
CTCGACTTCTTTTGGAAATATCTTGAAGAAAAATATGAAGAGCATTTGTA
TGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGT
TTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAA
CACAGTCTATGGCCATCATACTGTTATATAGCTGACAAGCACAAACATGTT
GGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCG
GTTTTGGATATTAGATACGGTGTTCGAGAATTGCATATAGTAAAGACT
TTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAA
AATGTTCTGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCAT
GTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTAT
ACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAA
AAAACGTATTGAAGCTATCCACAAATTGATAAGTACTTGAAATCCAGC
AAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTG
GCGACCATCCTCCAAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCT
GGGATCCGCTCGGCCGCTAGTGCCAGCTCGCAGAAGGCGCTGCTGCTG
GAGCTCAAGGGGCTGCAGGAAGAGCCGGTCGAGGGATTCCGCGTGACAC
TGGTGGACGAGGGCGATCTATACAACTGGGAGGTGGCCATCTTCGGGCC
CCCCAACACCTACTACGAGGGCGGCTACTTCAAGGCGCGCCTCAAGTTC
CCATCGACTACCCATACTCTCCACCAGCCTTTCGGTTCCTGACCAAGA
TGTGGCACCCTAACATCTACGAGACGGGGGACGTGTGTATCTCCATCCT
CCACCCGCCGGTGGACGACCCCCAGAGCGGGGAGCTGCCCTCAGAGAGG
TGGAACCCACGCAGAACGTCAGGACCATTCTCCTGAGTGTGATCTCCC
TCCTGAACGAGCCCAACACCTTCTCGCCCGCAAACGTGGACGCCTCCGT
GATGTACAGGAAGTGGAAGAGAGCAAGGGGAAGGATCGGGAGTACACA
GACATCATCCGGAAGCAGGTCCTGGGACCAAGGTGGACGCGGAGCGTGA
CGGCGTGAAGGTGCCACACGCTGGCCGAGTACTGCGTGAAGACCAAG
GCGCCGGCGCCCGACGAGGGCTCAGACCTCTTCTACGACGACTACTACG
AGGACGGCGAGGTGGAGGAGGAGGCCGACAGCTGCTTCGGGGACGATGA
GGATGACTCTGGCACGGAGGAGTCCtgagcgggccgc