

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of SNX3 [1 – 162]

<u>Enzyme description:-</u>	SNX3 [1 – 162]
<u>Clone number:-</u>	DU 61034
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i> ,
<u>Tag:-</u>	N-terminal His(6) - SUMO
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Calculated molecular mass:-</u>	
Monoisotopic	30, 737.58 daltons
Average Mass	30, 756.73 daltons
	[cysteines reduced, methionines have not been oxidised]
<u>Theoretical pI:-</u>	6.32
<u>Purity:-</u>	>80 %
<u>Enzyme storage buffer:-</u>	50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF
<u>Storage temperature:-</u>	-70 °C

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Clone Data Sheet

SNX3 [1 - 162]

Protein SNX3 [1 - 162]

Clone number DU 61034

Species Human

Accession number NM_003795.6

Tags N-terminal His(6) + SUMO

**Bacterially
expressed SNX3
protein**

MGHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFVKVMT
THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE
VYQEQTGG**MAETVADTRRLITKPQNLNDAYGPPSNFLEIDVSNPQTVG**
VGRGRFTTYEIRVKTNLPIFKLKESTVRRRYSDFEWLRSSELERESKVV
VPPLPGKAFLRQLPFRGDDGIFDDNFIEERKQGLEQFINKVAGHPLAQ
NERCLHMFLQDEIIDKSYTPSKIRHA

Native sequence Amino acids M1 – A162 (end) of human SNX3.
Residue M105 of the fusion protein is equivalent to M1 of the native
enzyme. The His(6) tag is located at residues 2 – 7.

Protease cleavage SENP1 cleavage of SUMO:
(SDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFVKVMTT
HLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGME
EEDVIEVYQEQTGG) residues 9 - 104

Cloning sites *Bam*H1 and *Not*1 sites of pET15b His6-SUMO

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Complete Nucleotide Sequence

ATGGGTCATCATCACCATCACCATTCTGACCAGGAGGCAAAACCTTCA
ACTGAGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAACCTCAA
GTCATTGGACAGGATAGCAGTGAGATTCACTTCAAAGTGCAAATGACA
ACACATCTCAAGAACTCAAAGAATCATACTGTCAAAGACAGGGTGTT
CCAATGAATTCCTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGAT
AATCATACTCCAAAAGAAGCTGGGAATGGAGGAAGAAGATGTGATTGAA
GTTTATCAGGAACAAACGGGGGAATGGCGGAGACCGTGGCTGACACC
CGGCGGCTGATCACCAAGCCGCAGAACCTGAATGACGCCTACGGACCC
CCCAGCAACTTCTCGAGATCGATGTGAGCAACCCGCAAACGGTGGGG
GTCGGCCGGGGCCGCTTCACCACTTACGAAATCAGGGTCAAGACAAAT
CTTCTATTTTTCAAGCTGAAAGAATCTACTGTTAGAAGAAGATACAGT
GACTTTGAATGGCTGCGAAGTGAATTAGAAAGAGAGCAAGGTCGTA
GTTCCCCGCTCCCTGGGAAAGCGTTTTTGCCTCAGCTTCCTTTTAGA
GGAGATGATGGAATATTTGATGACAATTTTATTGAGGAAAGAAAACAA
GGGCTGGAGCAGTTTATAACAAGGTCGCTGGTCATCCTCTGGCACAG
AACGAACGTTGTCTTCACATGTTTTTACAAGATGAAATAATAGATAAA
AGCTATACTCCATCTAAAATAAGACATGCctga