

*Division of Signal Transduction Therapy*

**Standard Operation Procedure**

**Preparation of GST-NEDD8**

**Enzyme description:-** GST-NEDD8 1-76 (mature form)

**Clone number:-** DU32162

**Source:-** human recombinant

**Tag:-** N-terminal GST

**Purification method:-** GSH-Sepharose

**Expression system:-** E.coli

**Calculated molecular mass:-**

Monoisotopic 35361 Da  
Average Mass 35383 Da  
[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 6.0

**Purity:-** 90%

**Enzyme storage buffer:-**

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

**Storage temperature:-** -80°C

**Assay:-**

Loading assay with UBE2M or UBE2F

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### **Clone Data Sheet**

#### **GST-NEDD8 1-76**

<b><u>Protein</u></b>	NEDD8 (1-76 = mature full length)
<b><u>Synonyms</u></b>	Ubiquitin-like protein NEDD8
<b><u>Clone Number</u></b>	DU32162
<b><u>Species</u></b>	Human
<b><u>Accession Number</u></b>	Protein: Q15843
<b><u>Tags</u></b>	N-terminal GST
Aminoacid sequence of the expressed protein	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGL EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAESMLEGAVL DIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTH PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIA WPLQGWQATFGGGDHPPKSD <u>LEVLFQGPLGSMLIKVKTLTGKEIEIDIEP</u> <b>TDKVERIKERVEEKEGIPPOQR</b> LIYSGKQMNDEKTAADYKILGGSVLHL <b>VLALRGG</b>
Native sequence	The native sequence is 4 aa longer. These are normally cleaved off by SENP8 / DEN1 protease to obtain mature NEDD8
Protease cleavage	Precession protease site underlined
Cloning sites	BamHI / NotI
<b><u>DNA sequence of insert</u></b>	GGATCCATGCTAATTAAAGTGAAGACGCTGACCGGAAAGGAGATTGAGAT TGACATTGAACCTACAGACAAGGTGGAGCGAATCAAGGAGCGTGTGGAGG AGAAAGAGGGAATCCCCCACAACAGCAGAGGCTCATCTACAGTGGCAAG CAGATGAATGATGAGAAGACAGCAGCTGATTACAAGATTTTAGGTGGTTC AGTCCTTACCTGGTGTGGCTCTGAGAGGAGGATAAGGTCTTAGGCAGT GAGCGGCCGC