

## *Division of Signal Transduction Therapy*

### Standard Operation Procedure

#### Preparation of NEDD8

<b><u>Enzyme description:-</u></b>	NEDD8
<b><u>Clone number:-</u></b>	DU23245
<b><u>Source:-</u></b>	human recombinant
<b><u>Tag:-</u></b>	no tag
<b><u>Purification method:-</u></b>	refolded from inclusion bodies, IEX & SEC
<b><u>Expression system:-</u></b>	E.coli
<b><u>Calculated molecular mass:-</u></b>	
Monoisotopic	8554 Da
Average Mass	8559 Da
[cysteines reduced, methionines have not been oxidised]	
<b><u>Theoretical pI:-</u></b>	7.7
<b><u>Purity:-</u></b>	90%
<b><u>Enzyme storage buffer:-</u></b>	
50mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT	
<b><u>Storage temperature:-</u></b>	-80°C
<b><u>Assay:-</u></b>	
Loading assay with UBE2M or UBE2F	

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**Clone Data Sheet**

**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>	DU23245
<b><u>Species</u></b>	Human
<b><u>Accession Number</u></b>	Protein: Q15843
<b><u>Tags</u></b>	no tag
Aminoacid sequence of the expressed protein	<b>MLIKVKTLTGKEIEIDIEPTDKVERIKERVEEKEGIPPOQORLIYSG KQMNDEKTAADYKILGGSVLHLVLALRGG</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	N/A
Cloning sites	NcoI / BamHI
<b><u>DNA sequence of insert</u></b>	<b>CCATGCTAATTAAAGTGAAGACGCTGACCGGAAAGGAGATTGAGATT GACATTGAACCTACAGACAAGGTGGAGCGAATCAAGGAGCGTGTGGA GGAGAAAGAGGGAATCCCCCACAACAGCAGAGGCTCATCTACAGTG GCAAGCAGATGAATGATGAGAAGACAGCAGCTGATTACAAGATTTA GGTGGTTCAGTCCTTCACCTGGTGTGGCTCTGAGAGGAGGATAAGG ATCC</b>