

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of NEDD4 H865A C867A [1 – 900]

Enzyme description:- NEDD4 H865A C867A [1 - 900]

Clone number:- DU 79134

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Agarose

Calculated molecular mass:-

Monoisotopic 130, 860.74 daltons

Average Mass 130, 943.06 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.66

Purity:- >85 %

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

NEDD4 H865A C867A
[1 - 900]

Protein NEDD4 H865A C867A [1 - 900]

Clone number DU 79134

Species Human

Accession number P46934-4

Tags N-terminal GST

**Bacterially
expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVL FQG PLGSMATCAVEVFGLLED
EENSRIVRVRVIAGIGLAKKDILGASDPYVRVTLYDPMNGVLT SVQTKT
IKKSLNPKWNEEILFRVHPQQRLLFEVFDENRLTRDDFLGQVDVPLYP
LPTENPRLERPYTFKDFVLHPRSHKSRVKGYLR LKMTYLPKTS GSEDDN
AEQAEELPGWVVDQPDAAACHLQQQQE PSPLPPGWEERQDILGRYYV
NHESRRTQWKRPTPDNL TDAENGNIQLQAQRAFTTRRQISEETESVDN
RESSENWEI IREDEATMYSNQAFSPSPSSNLDVPTH LAEELNARLTI F
GNSAVSQPASSSNHSSRRGSLQAYTFEEQPTLPVLLPTSSGLPPGWE E
QDERGRSYVDHNSRRTTTWTKPTVQATVETSQLTSSQSSAGPQSQASTS
DSGQQVTQPSEIEQGFLPKGWEVRHAPNGRPF FIDHNTKTTTWEDPRLK
IPAHLRGKTSLDTSNDLGPLPPGWEERTHTDGRIFYINHNIKRTQWEDP
RLENVAITGPAVPYSRDYKRKYEFFRRKLKKQNDI PNKFEMKLRRATVL
EDSYRRIMGVKRADFLKARLWIEFDGEKGLDYGGVAREWFFLI SKEMFN
PYYGLFEYSATDNYTLQINPNSGLCNE DHLSYFKF IGRVAGMAVYHGKL
LDGFFIRPFYKMLHKPITLHDMESVDSEYYNSLRWILENDPTELDLRF
IIDEELFGQTHQHELKNGGSEIVVTNKNKKEYIYLVIQWR FVNRIQKQM
AAFKEGFFELIPQDLIKIFDENELELLMCGLGDVDVNDWREHTKYKNGY
SANHQVIQWFWKAVLMM DSEKRIRLLQFVTGTSRVPMNGFAELYGSNGP
QSFTVEQWGTPEKLPRAATAFNRLDLPPYESFEELWDKLQMAIENTQGF
DGVD

Native sequence Amino acids M1 – D900 (end) of human NEDD4.
Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

The enzyme has H865A and C867A catalytic inactive mutations.
Residues H865 and C867 are equivalent to A1096 and A1098 of the fusion protein.

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Protease cleavage PreScission (LEVLFQGP) residues 221 - 229

Cloning sites *Bam*HI and *Not*I site of pGex6P-1