

MRC PPU REAGENTS

Standard Operating Procedure

Preparation of RNF115 W259A [1 – 305]

Enzyme description:- RNF115 W259A [1 - 305]

Clone number:- DU 51992

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 60, 902.80 daltons

Average Mass 60, 941.85 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.53

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270mM 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

RNF115 W259A [1 - 305]

Protein RNF115 W259A [1 - 305]

Clone number DU 51992

Species Mouse

Accession number Q9D0C1.1

Tags N-terminal GST

**Bacterially
expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA
VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVL FQG PLGSPEF**MAEASAAGADA**
GSVA AHRFFCHFCKEVNPKLPEYICPRCDSGFIEEVTDSSFLGGGG
SRTDNSTATHFAELWDHLDHTMFLQDFRPFLSSNPLDQDNRANERGHQT
HTDFWGPSRPPRLPMTRRYRSRGSTRPDRSPAIEGIIQQIFAGFFANSA
IPGSPHPFSWSGMLHSNPGDYAWGQTGLDAIVTQLLGQLENTGPPPADK
EKITS LPTVTVTQEQVNTGLECPVCKEDYTVEEKVRQLPCNHFFHSSCI
VPALLELHDTCPVCRKSLNGEDSTRQTQSSEASASNRFNSNDSQLHDRWTF

Native sequence Amino acids M1 – F305 (end) of mouse RNF115.
Residue M235 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

The enzyme has a W259**A** mutation. Residue W259 is equivalent to **A493** of the fusion protein.

Protease cleavage PreScission (LEVL FQGP) residues 221 – 228.

Cloning sites *Eco*R1 and *Not*I site of pGex6P1

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Nucleotide
Sequence of insert

gaattcATGGCGGAGGCCTCGGCGGCGGGGGCGGACGCGGGCTC
CGCTGTCCGCGCACCGTTTTCTTCTGCCACTTTTGTAAGGGCG
AGGTCAATCCCAAACCTACCGGAATATATATGTCCCAGATGTGAC
TCAGGCTTTATTGAGGAAGTGACAGATGATTCCAGTTTTTTAGG
TGGTGGTGGAAAGCCGGACAGACAATAGCACAGCGACACATTTTG
CAGAGCTTTGGGACCATCTGGATCACACAATGTTTTTACAAGAT
TTTAGACCATTTCTAAGTAGCAATCCACTGGACCAAGATAATAG
AGCCAATGAGAGAGGTCACCAAACCTCACACTGACTTTTGGGGAC
CAAGTCGGCCTCCAAGGTTGCCAATGACAAGAAGATACAGGTCT
CGCGGAAGTACTCGTCCCGACAGGTCGCCAGCTATCGAAGGAAT
AATACAACAGATCTTTGCAGGATTTCTTTGCAAATTTCTGCCATTC
CTGGATCCCCACACCCCTTTTCTTGGAGCGGGATGCTGCACTCC
AACCCTGGGGACTATGCCTGGGGTCAGACAGGCCTTGATGCCAT
TGTAACCCAGCTTCTAGGACAGCTGGAAAACACGGGTCCCCCTC
CAGCCGACAAGGAGAAGATCACATCTCTCCAACAGTTACAGTA
ACTCAGGAACAAGTCAATACGGGTTTAGAATGTCCAGTATGCAA
AGAAGATTACACAGTTGAGGAGAAAGTCCGGCAGTTACCCTGCA
ACCACTTCTTTCACAGCAGCTGCATCGTGCCCGCGTTAGAAGT
CATGACACATGCCAGTATGTAGGAAGAGCTTAAATGGTGAGGA
CTCTACTCGGCAAACCCAGAGCTCCGAGGCCTCTGCAAGCAACA
GATTTAGCAATGACAGCCAGCTACATGACCGATGGACTTTCtga
gcggccgc