MRC PPU REAGENTS

Standard Operating Procedure

Preparation of RNF115 [1 – 305]

Enzyme description:- RNF115 [1 - 305]

Clone number:- DU 51967

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 61, 017.85 daltons
Average Mass 61, 056.99 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 [1 - 305]

Protein	RNF115 [1 - 305]
Clone number	DU 51967
Species	Mouse
Accession number	Q9D0C1.1
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEF**MAEASAAGADA** **GSAVAAHRFFCHFCKGEVNPKLPEYICPRCDSGFIEEVTDDSSFLGGGG** **SRTDNSTATHFAELWDHLDHTMFLQDFRPFLSSNPLDQDNRANERGHQT** **HTDFWGPSRPPRLPMTRRYRSRGSTRPDRSPAIEGIIQQIFAGFFANSA** **IPGSPHPFSWSGMLHSNPGDYAWGQTGLDAIVTQLLGQLENTGPPPADK** **EKITSLPTVTVTQEQVNTGLECPVCKEDYTVEEKVRQLPCNHFFHSSCI** **VPWLELHDTCPVCRKSLNGEDSTRQTQSSEASASNRFSNDSQLHDRWTF**
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.
Protease cleavage	PreScission (LEVLFQGP) residues 221 – 228.
Cloning sites	*Eco*R1 and *Not*1 site of pGex6P1

Nucleotide Sequence of insert

gaattcATGGCGGAGGCCTCGGCGGCGGGGGCGGACGCGGGCTC
CGCTGTCGCCGCGCACCGTTTCTTCTGCCACTTTTGTAAGGGCG
AGGTCAATCCCAAACTACCGGAATATATATGTCCCAGATGTGAC
TCAGGCTTTATTGAGGAAGTGACAGATGATTCCAGTTTTTTAGG
TGGTGGTGGAAGCCGGACAGACAATAGCACAGCGACACATTTTG
CAGAGCTTTGGGACCATCTGGATCACACAATGTTTTTACAAGAT
TTTAGACCATTTCTAAGTAGCAATCCACTGGACCAAGATAATAG
AGCCAATGAGAGAGGTCACCAAACTCACACTGACTTTTGGGGAC
CAAGTCGGCCTCCAAGGTTGCCAATGACAAGAAGATACAGGTCT
CGCGGAAGTACTCGTCCCGACAGGTCGCCAGCTATCGAAGGAAT
AATACAACAGATCTTTGCAGGATTCTTTGCAAATTCTGCCATTC
CTGGATCCCCACACCCCTTTTCTTGGAGCGGGATGCTGCACTCC
AACCCTGGGGACTATGCCTGGGGTCAGACAGGCCTTGATGCCAT
TGTAACCCAGCTTCTAGGACAGCTGGAAAACACGGGTCCCCCTC
CAGCCGACAAGGAGAAGATCACATCTCTCCCAACAGTTACAGTA
ACTCAGGAACAAGTCAATACGGGTTTAGAATGTCCAGTATGCAA
AGAAGATTACACAGTTGAGGAGAAAGTCCGGCAGTTACCCTGCA
ACCACTTCTTTCACAGCAGCTGCATCGTGCCGTGGTTAGAACTG
CATGACACATGCCCAGTATGTAGGAAGAGCTTAAATGGTGAGGA
CTCTACTCGGCAAACCCAGAGCTCCGAGGCCTCTGCAAGCAACA
GATTTAGCAATGACAGCCAGCTACATGACCGATGGACTTTCtga
gcggccgc