MRC PPU REAGENTS

Standard Operating Procedure

Preparation of RNF115 [1 – 305]

Enzyme description:- RNF115 [1 - 305]

Clone number:- DU 56208

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal MBP

Purification method:- Amylose Resin

Calculated molecular mass:-
Monoisotopic 78, 610.64 daltons
Average Mass 78, 659.98 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.06

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 56208
Species	Mouse
Accession number	Q9D0C1.1
Tags	N-terminal MBP
Bacterially expressed protein	MMKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKF PQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDA VRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKS ALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTF LVDLIKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYG VTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEA VNKDKPLGAVALKSYEEELVKDPRIAATMENAQKGEIMPNIPQMSAFWY AVRTAVINAASGRQTVDEALKDAQTNSSSNNNNNNNNNNLGDDDDKVPE FLEVLFQGPLGSPGEF**MAEASAAGADAGSAVAAHRFFCHFCKGEVNPKL** **PEYICPRCDSGFIEEVTDDSSFLGGGGSRTDNSTATHFAELWDHLDHTM** **FLQDFRPFLSSNPLDQDNRANERGHQTHTDFWGPSRPPRLPMTRRYRSR** **GSTRPDRSPAIEGIIQQIFAGFFANSAIPGSPHPFSWSGMLHSNPGDYA** **WGQTGLDAIVTQLLGQLENTGPPPADKEKITSLPTVTVTQEQVNTGLEC** **PVCKEDYTVEEKVRQLPCNHFFHSSCIVPWLELHDTCPVCRKSLNGEDS** **TRQTQSSEASASNRFSNDSQLHDRWTF**
Native sequence	Amino acids M1 – F305 (end) of mouse RNF115. Residue M409 of the fusion protein is equivalent to M1 of the native enzyme. The MBP tag is located at residues 1 – 393.
Protease cleavage	PreScission (LEVLFQGP) residues 394 – 401.
Cloning sites	*Eco*R1 and *Not*1 site of pMEX3C

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