

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of PreScission Protease Alfa Tag

Enzyme description:- PreScission Protease Alfa Tag

Clone number:- DU 67332

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal GST

Purification method:- GSH-Sepharose

Calculated molecular mass:-

Monoisotopic 48, 138.77 daltons

Average Mass 48, 169.55 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.81

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 % 2-mercaptoethanol

Storage temperature:- -70 °C

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Clone Data Sheet

PreScission Protease Alfa Tag

<u>Protein</u>	PreScission Protease Alfa Tag
<u>Clone number</u>	DU 67332
<u>Species</u>	Human
<u>Accession number</u>	NP_740524.1
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKERAIEISMLE GAVLDIRYGVSRIAYSKDFETLKVDLFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY LKSSKYIAWPLQGWQATFGGGDHPKSDLVPRGSGGGPNTEFALSLLR KNIMTITTSKGEFTGLGIHDRVCVIPPTHAQPGDDVLVNGQKIRVKDKY KLVDPENINLELTVLTLDRNEKFRDIRGFISEDLEGVDATLVVHSNNF TNTILEVGPVTMAGLINLSSTPTNRMIRYDYATKTGCGGVLCATGKI FGIHVGGNGRQGFSAQLKKQYFVEKQPSRLEEELRRRLTE</p>
<u>Native sequence</u>	<p>Amino acids G1 – Q182 (end) of human Rhinovirus B14. Residue G229 of the fusion protein is equivalent to G1538 of the native enzyme. The Alfa tag is located at residues 405 – 424. The GST tag is located at residues 1 - 220.</p>
<u>Protease cleavage</u>	Thrombin (LVPRGS) residues 221 - 226
<u>Cloning sites</u>	<i>Kpn</i> 1 and <i>Not</i> 1 sites of pGEX4T-1

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Nucleotide Sequence

ATGTCCCCTATACTAGGTTATTGGAAAAATTAAGGGCCTTGTGCAACCC
ACTCGACTTCTTTTGGGAATATCTTGAAGAAAAATATGAAGAGCATTG
TATGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTG
GGTTTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAA
TTAACACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAAAC
ATGTTGGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAA
GGAGCGGTTTTGGATATTAGATACGGTGTTCGAGAATTGCATATAGT
AAAGACTTTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAA
ATGCTGAAAATGTTCAAGATCGTTTATGTCATAAAACATATTTAAAT
GGTGATCATGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGAT
GTTGTTTTTATACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTA
GTTTGTTTTTAAAAAACGTATTGAAGCTATCCCACAAATTGATAAGTAC
TTGAAATCCAGCAAGTATATAGCATGGCCTTTCAGGGCTGGCAAGCC
ACGTTTGGTGGTGGCGACCATCCTCCAAAATCGGATCTGGTTCCGCGT
GGATCCGGTGGAGGACCAAACACAGAATTTGCACTATCCCTGTTAAGG
AAAAACATAATGACTATAACAACCTCAAAGGGAGAGTTCACAGGGTTA
GGCATAACATGATCGTGTCTGTGTGATACCCACACACGCACAGCCTGGT
GATGATGTACTAGTAAATGGTCAGAAAATTAGAGTTAAGGATAAGTAC
AAATTAGTAGATCCAGAGAACATTAATCTAGAGCTTACAGTGTGACT
TTAGATAGAAATGAAAAATTCAGAGATATCAGGGGATTTATATCAGAA
GATCTAGAAGGTGTGGATGCCACTTTGGTAGTACATTCAAATAACTTT
ACCAACACTATCTTAGAAGTTGGCCCTGTAACAATGGCAGGACTTATT
AATTTGAGTAGCACCCCCACTAACAGAATGATTCGTTATGATTATGCA
ACAAAAACTGGGCAGTGTGGAGGTGTGCTGTGTGCTACTGGTAAGATC
TTTGGTATTCATGTTGGCGGTAATGGAAGACAAGGATTTTCAGCTCAA
CTTAAAAACAATATTTTGTAGAGAAACAACCTAGTCGTCTAGAAGAG
GAACTGCGTCGCCGACTGACGGAAtga