

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of PreScission Protease

Enzyme description:- PreScission Protease

Clone number:- DU 2328

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 76, 915.91 daltons

Average Mass 76, 965.88 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.73

Purity:- >80 %

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

Division of Signal Transduction Therapy

Clone Data Sheet

PreScission Protease

<u>Protein</u>	PreScission Protease
<u>Clone number</u>	DU 2328
<u>Species</u>	Human
<u>Accession number</u>	GNNYH4
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLVPRGSGGGPNTEFALSLLRKNIMT ITTSKGEFTGLGIHDRVCVIPTHAQPGDDVLVNGQKIRVKDKYKLVDP NINLELTVLTLDRNEKFRDIRGFISEDLEGVDATLVVHSNNFTNTILEV GPVTMAGLINLSSPTNRMIRYDYATKTGQCGGVLCATGKIFGIHVGGN GRQGFSAQLKKQYFVEKQ</p>
<u>Native sequence</u>	Amino acids G1538 – Q1719 (end F2179) of human Rhinovirus 14. Residue G229 of the fusion protein is equivalent to G1538 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	Thrombin (<u>LVPRGS</u>) residues 221 - 226
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Eco</i> R1 sites of pGEX-4T

Division of Signal Transduction Therapy

Complete Nucleotide Sequence

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCA
CTCGACTTCTTTTGGAAATATCTTGAAGAAAAATATGAAGAGCATTGTA
TGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGT
TTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAA
CACAGTCTATGGCCATCATAACGTTATATAGCTGACAAGCACAACATGTT
GGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCG
GTTTTGGATATTAGATACGGTGTTCGAGAATTGCATATAGTAAAGACT
TTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAA
AATGTTTCGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCAT
GTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTAT
ACATGGACCCAATGTGCCTGGATGCGTTCCCAAATTAGTTTGTTTTAA
AAAACGTATTGAAGCTATCCACAAATTGATAAGTACTTGAAATCCAGC
AAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTG
GCGACCATCCTCCAAAATCGGATCTGGTCCGCGTGGATCCGGTGGAGG
ACCAAACACAGAATTTGCACTATCCCTGTTAAGGAAAAACATAATGACT
ATAACAACCTCAAAGGGAGAGTTCACAGGGTTAGGCATACATGATCGTG
TCTGTGTGATACCCACACACGCACAGCCTGGTGATGATGTACTAGTAAA
TGGTCAGAAAATTAGAGTTAAGGATAAGTACAAATTAGTAGATCCAGAG
AACATTAATCTAGAGCTTACAGTGTGACTTTAGATAGAAATGAAAAAT
TCAGAGATATCAGGGGATTTATATCAGAAGATCTAGAAGGTGTGGATGC
CACTTTGGTAGTACATTCAAATAACTTTACCAACACTATCTTAGAAGTT
GGCCCTGTAACAATGGCAGGACTTATTAATTTGAGTAGCACCCCCACTA
ACAGAATGATTCGTTATGATTATGCAACAAAAACTGGGCAGTGTGGAGG
TGTGCTGTGTGCTACTGGTAAGATCTTTGGTATTCATGTTGGCGGTAAT
GGAAGACAAGGATTTTCAGCTCAACTTAAAAACAATATTTTGTAGAGA
AACAAatga