

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of PreScission Protease**

**Enzyme description:-** PreScission Protease

**Clone number:-** DU 34905

**Source:-** Recombinant

**Expression system:-** *E.coli*

**Tag:-** N-terminal His6

**Purification method:-** Ni<sup>2+</sup>-NTA agarose

**Calculated molecular mass:-**

Monoisotopic 23, 639.99 daltons

Average Mass 23, 654.90 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 9.01

**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

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**Clone Data Sheet**

**PreScission Protease**

<b><u>Protein</u></b>	PreScission Protease
<b><u>Clone number</u></b>	DU 34905
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	GNNYH4
<b><u>Tags</u></b>	N-terminal GST
<b><u>Bacterially expressed protein</u></b>	<b>MGSSHHHHHSSGLVPRGSHMASMTGGQQMGRGSGGGPNT<b>EFALSLLRK</b> N<b>IMTITTSKGEFTGLGIHDRVCVIP</b>THA<b>QPGDDVLVNGQKIRVKDKYKL</b> V<b>DPENINLELTVLTLDRNEKFRDIRGF</b>ISED<b>LEGVDATLVVHSNNFTNT</b> I<b>LEVGPVTMAGLINLSSTPTNRMIRYDYATKTGQCGGVLCATGKIFGIH</b> V<b>GGNGRQGFSAQLKKQYFVEKQ</b></b>
<b><u>Native sequence</u></b>	Amino acids G1538 – Q1719 (end F2179) of human Rhinovirus 14. Residue G37 of the fusion protein is equivalent to G1538 of the native enzyme. The His6 tag is located at residues 5 – 10.
<b><u>Protease cleavage</u></b>	Thrombin ( <u>LVPRGS</u> ) residues 14 – 19.
<b><u>Cloning sites</u></b>	<i>Bam</i> H1 and <i>Sal</i> I sites of pET28a

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**Nucleotide**  
**Sequence Of**  
**Insert**

ggatccgGTGGAGGACCAAACACAGAATTTGCACTATCCCTGTTAAGGA  
AAACATAATGACTATAACAACCTCAAAGGGAGAGTTCACAGGGTTAGG  
CATACATGATCGTGTCTGTGTGATACCCACACACGCACAGCCTGGTGAT  
GATGTACTAGTAAATGGTCAGAAAATTAGAGTTAAGGATAAGTACAAAT  
TAGTAGATCCAGAGAACATTAATCTAGAGCTTACAGTGTTGACTTTAGA  
TAGAAATGAAAAATTCAGAGATATCAGGGGATTTATATCAGAAGATCTA  
GAAGGTGTGGATGCCACTTTGGTAGTACATTCAAATAACTTTACCAACA  
CTATCTTAGAAGTTGGCCCTGTAACAATGGCAGGACTTATTAATTTGAG  
TAGCACCCCACTAACAGAATGATTCGTTATGATTATGCAACAAAACT  
GGCAGTGTGGAGGTGTGCTGTGTGCTACTGGTAAGATCTTTGGTATTC  
ATGTTGGCGGTAATGGAAGACAAGGATTTTCAGCTCAACTTAAAAACA  
ATATTTTGTAGAGAAACAAtgagtcgac