*Division of Signal Transduction Therapy*

# Standard Operating Procedure

## Preparation of USP35 [1 - 1018]

**Enzyme description:-** USP35 [1 – 1018]

**Clone number:-** DU 25784

**Source:-** Recombinant

**Expression system:-** Baculovirus expression vector system

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**
Monoisotopic 140, 139.94 daltons
Average Mass 140, 229.19 daltons
[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 5.48

**Purity:-** >80 %

**Activation protocol:-** Constitutively active

**Enzyme storage buffer:-**
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

**Storage temperature:-** -70 °C

**Assay buffer:-**
50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2–mercaptoethanol, 10 mM MgAc

# Clone Data Sheet

## USP35 [1 - 1018]

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|---|---|
| **Protein** | USP35 [1 - 1018] |
| **Clone number** | DU 25784 |
| **Species** | Human |
| **Accession number** | Q9P2H5 |
| **Tags** | N-terminal GST |
| **Baculovirus expressed protein** | MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL<br>GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE<br>GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN<br>GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY<br>LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MDKILEAVV**<br>**TSSYPVSVKQGLVRRVLEAARQPLEREQCLALLALGARLYVGGAEELP**<br>**RRVGCQLLHVAGRHHPDVFAEFFSARRVLRLLQGGAGPPGPRALACVQ**<br>**LGLQLLPEGPAADEVFALLRREVLRTVCERPGPAACAQVARLLARHPR**<br>**CVPDGPHRLLFCQQLVRCLGRFRCPAEGEEGAVEFLEQAQQVSGLLAQ**<br>**LWRAQPAAILPCLKELFAVISCAEEEPPSSALASVVQHLPLELMDGVV**<br>**RNLSNDDSVTDSQMLTAISRMIDWVSWPLGKNIDKWIIALLKGLAAVK**<br>**KFSILIEVSLTKIEKVFSKLLYPIVRGAALSVLKYMLLTFQHSHEAFH**<br>**LLLPHIPPMVASLVKEDSNSGTSCLEQLAELVHCMVFRFPGFPDLYEP**<br>**VMEAIKDLHVPNEDRIKQLLGQDAWTSQKSELAGFYPRLMAKSDTGKI**<br>**GLINLGNTCYVNSILQALFMASDFRHCVLRLTENNSQPLMTKLQWLFG**<br>**FLEHSQRPAISPENFLSASWTPWFSPGTQQDCSEYLKYLLDRLHEEEK**<br>**TGTRICQKLKQSSSPSPPEEPPAPSSTSVEKMFGGKIVTRICCLCCLN**<br>**VSSREEAFTDLSLAFPPPERCRRRRLGSVMRPTEDITARELPPPTSAQ**<br>**GPGRVGPRRQRKHCITEDTPPTSLYIEGLDSKEAGGQSSQEERIEREE**<br>**EGKEERTEKEEVGEEEESTRGEGEREKEEEVEEEEEKVEKETEKEAEQ**<br>**EKEEDSLGAGTHPDAAIPSGERTCGSEGSRSVLDLVNYFLSPEKLTAE**<br>**NRYYCESCASLQDAEKVVELSQGPCYLILTLLRFSFDLRTMRRRKILD**<br>**DVSIPLLLRLPLAGGRGQAYDLCSVVVHSGVSSESGHYYCYAREGAAR**<br>**PAASLGTADRPEPENQWYLFNDTRVSFSSFESVSNVTSFFPKDTAYVL**<br>**FYRQRPREGPEAELGSSRVRTEPTLHKDLMEAISKDNILYLQEQEKEA**<br>**RSRAAYISALPTSPHWGRGFDEDKDEDEGSPGGCNPAGGNGGDFHRLV**<br>**F** |
| **Native sequence** | Amino acids M1 – F1018 (end) of human USP35.<br>Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220. |

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| **Protease cleavage** | PreScission site (LEVLFQGP) residues 221 – 228 |
| **Cloning sites** | *Bam*H1 and *Not*1 sites of pFastBac GST 6P1 |

**Nucleotide sequence of insert**

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ggatccATGGACAAGATCTTGGAGGCGGTGGTGACGTCGTCATACCCG
GTCAGCGTGAAGCAGGGGCTGGTTCGGCGCGTGCTGGAGGCGGCGCGG
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GAAGGAGAGAGGGAGAAAGAGGAGGAGGTGGAAGAGGAAGAAGAGAAG
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