

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of PTIP [590 - 1069]

Enzyme description:- PTIP [590 – 1069]

Clone number:- DU 5838

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 81, 487.08 daltons

Average Mass 81, 539.74 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 7.95

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, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 deg C

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Clone Data Sheet

PTIP [590 – 1069]

Protein PTIP [590 – 1069]

Clone number DU 5838

Species Human

Accession number NM_007349.3

Tags N-terminal GST

Bacterially expressed protein MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKL TQSM A I RY I ADKHNMLGGCPKERA E I SMLE
GAVLDIRYGVSRIAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSD LEVLFQGP LGSPEF **QLFGHD**
PAVEIPEEGFLLGCVFAIADYPEQMSDKQLLATWKRI IQAHGGTVDPT
FTSRCTHLLCESQVSSAYAQAIRERKRCVTAHWLNTVLKKKKMVP
PHRALHFPVAFPPGGKPCSQHI ISVTGFVDSDRDDLKLMAYLAGAKYTGYL
CRSNTVLICKEPTGLKYEKAKEWRI PCVNAQWLGDILLGNFEALRQIQ
YSRYTAFSLQDPFAPTQHLVLNLLDAWRVPLKVS AELLMSIRLPPKLK
QNEVANVQPSSKRARIEDVPPPTKKLTP ELTPFVLF TGFEPVQVQYI
KKLYILGGEVAESAQKCTHLIASKVTRTVKFLTAISVVKHIVTPEWLE
ECFRCQKFIDEQNYILRDAEAEVLF SFSLEESLKRAHVSPLFKAKYFY
ITPGICPSLSTMKAIVECAGGKVL SKQPSFRKLM EHKQNSSLSEI ILI
SCENDLHLCREYFARGIDVHNAEFVLTGVLTQTLDYESYKFN

Native sequence Amino acids Q590 – N1059 (end) of human PTIP.
Residue Q235 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFQGP) residues 221 – 228

Cloning sites *Eco*R1 and *Not*I site of pGEX6P-1

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**Nucleotide
sequence of
insert**

gaattcCAGCTTTTTGGACATGATCCAGCAGTGGAGATTCCAGAAGAA
GGCTTCTTATTGGGATGTGTGTTTGCAATTGCGGATTATCCAGAGCAG
ATGTCTGATAAGCAACTGCTGGCCACCTGGAAAAGGATAATCCAGGCA
CATGGCGGCACTGTTGACCCACCTTCACGAGTCGATGCACGCACCTT
CTCTGTGAGAGTCAAGTCAGCAGCGGTATGCACAGGCAATAAGAGAA
AGAAAGAGATGTGTTACTGCACACTGGTTAAACACAGTCTTAAAGAAG
AAGAAAATGGTACCGCCGCACCGAGCCCTTCACTTCCAGTGGCCTTC
CCACCAGGAGGAAAGCCATGTTACAGCATATTATTTCTGTGACTGGA
TTTGTGATAGTGACAGAGATGACCTAAAATTAATGGCTTATTTGGCA
GGTGCCAAATATACGGGTATCTATGCCGCAGCAACACAGTCCATC
TGTAAGAACCAACTGGTTTAAAGTATGAAAAGCCAAAGAGTGGAGG
ATACCCTGTGTCAACGCCAGTGGCTTGGCGACATTCTTCTGGGAAAC
TTTGAGGCACTGAGGCAGATTCAGTATAGTCGCTACACGGCATTTCAGT
CTGCAGGATCCATTTGCCCTACCCAGCATTTAGTTTTAAATCTTTTA
GATGCTTGGAGAGTTCCCTTAAAAGTGTCTGCAGAGTTGTTGATGAGT
ATAAGACTACCTCCAAAACCTGAAACAGAATGAAGTAGCTAATGTCCAG
CCTTCTTCCAAAAGAGCCAGAATTGAAGACGTACCACCTCCCCTAAA
AAGCTAACTCCAGAATTGACCCCTTTTGTGCTTTTCACTGGATTCGAG
CCTGTCCAGGTTCAACAGTATATTAAGAAGCTCTACATTCTTGGTGGAA
GAGGTTGCGGAGTCTGCACAGAAGTGCACACACCTCATTGCCAGCAAG
GTGACTCGCACCGTGAAGTTCTGACGGCGATTTCTGTGCGTGAAGCAC
ATAGTGACGCCAGAGTGGCTGGAAGAATGCTTCAGGTGTCAGAAGTTC
ATTGATGAGCAGAACTACATTCTCCGAGATGCTGAGGCAGAAGTACTT
TTCTCTTTCAGCTTGGAAGAATCCTTAAAACGGGCACACGTTTCTCCA
CTCTTTAAGGCAAAATATTTTTACATCACACCTGGAATCTGCCAAGT
CTTTCCACTATGAAGGCAATCGTAGAGTGTGCAGGAGGAAAGGTGTTA
TCCAAGCAGCCATCTTTCGGAAGCTCATGGAGCACAAGCAGAAGTCCG
AGTTTGTGCGGAAATAATTTTAAATATCCTGTGAAAATGACCTTCATTTA
TGCCGAGAATATTTTGCCAGAGGCATAGATGTTCAATGCAGAGTTC
GTTCTGACTGGAGTGCTCACTCAAACGCTGGACTATGAATCATATAAG
TTTAACTgagcggccgc