

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active Platelet-derived growth factor receptor, alpha polypeptide [551 – 1089]

<u>Enzyme description:-</u>	PDGFRA [551 - 1089]
<u>Clone number:-</u>	DU 63237
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH Sepharose

Calculated molecular mass:-

Monoisotopic 88, 197.12 daltons
Average Mass 88, 253.72 daltons
[cysteines reduced, methionines have not been oxidised]

<u>Theoretical pI:-</u>	5.30
<u>Purity:-</u>	>80 %
<u>Activation protocol:-</u>	Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 10 mM DTT, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

<u>Storage temperature:-</u>	-70 °C
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Assay Buffer:-

50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 10 mM DTT, 10 mM MgAc

Substrate:-

KKKKEEIYFFFG Final concentration: 300 uM

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Clone Data Sheet

PDGFRA [551 - 1089]

Protein PDGFRA [551 - 1089]

Clone number DU 63237

Species Human

Accession number NM_006206.5

Tags N-terminal GST

Baculovirus expressed protein

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNK
KFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKE
RAEISMLEGAVLDIRYGVSR IAYSKDFETLKVDFLSKLPEMLKM
FEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKL
VCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSD
LEVLFQGPLGSQKPRYEIRWRVIESISPDGHEYIYVDPMQLPYD
SRWEFPRDGLVLGRVLGSGAFGKVVEGTAYGLSRSQPV MKVAVK
MLKPTARSSEKQALMSELKIMTHLGPHLNIVNLLGACTKSGPIY
IITEYCFYGDLVNYLHKNRDSFLSHHPEKPKKELDIFGLNPADE
STRSYVILSFENNGDYMDMKQADTTQYVPMLEKEVSKYSIDIQR
SLYDRPASYSKKKSM LDSEVKNLLSDDNSEGLTLLDLLSFTYQVA
RGMEFLASKNCVHRDLAARNVLLAQGKIVKICDFGLARDIMHDS
NYVSKGSTFLPVKWMAPESIFDNLYTTLSDVWSYGILLWEIFSL
GGTPYPGMMVDSTFYNKIKSGYRMAKPDHATSEVYEIMVKCWNS
EPEKRPSFYHLSEIVENLLPGQYKKS YEKIHLDFLKSDHPAVAR
MRVDS DNAYIGVTYKNEEDKLKDWEGLDEQRLSADSGYIIPLP
DIDPVPEEEDLGKRN RHSSQTSEESA IETGSSSSTFIKREDETI
EDIDMMDDIGIDSSDLVEDSFL

Native sequence Amino acids Q551 – L1089 (end residue) of human PDGFRA.
Residue Q232 of the fusion protein is equivalent to Q551 of the native enzyme. The GST tag is located at residues 1 - 220.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *Bam*H1 and *Not*I sites of pFastBac Dual.

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Nucleotide

sequence of insert

ggatcccAGAAACCGAGGTATGAAATTTCGCTGGAGGGTCATTGAA
TCAATCAGCCCGGATGGACATGAATATATTTATGTGGACCCGATG
CAGCTGCCTTATGACTCAAGATGGGAGTTTCCAAGAGATGGACTA
GTGCTTGGTCGGGTCTTGGGGTCTGGAGCGTTTGGGAAGGTGGTT
GAAGGAACAGCCTATGGATTAAGCCGGTCCCAACCTGTCATGAAA
GTTGCAGTGAAGATGCTAAAACCCACGGCCAGATCCAGTGAAAAA
CAAGCTCTCATGTCTGAACTGAAGATAATGACTCACCTGGGGCCA
CATTTGAACATTGTAAACTTGCTGGGAGCCTGCACCAAGTCAGGC
CCCATTTACATCATCACAGAGTATTGCTTCTATGGAGATTTGGTC
AACTATTTGCATAAGAATAGGGATAGCTTCCTGAGCCACCACCCA
GAGAAGCCAAAGAAAGAGCTGGATATCTTTGGATTGAACCCTGCT
GATGAAAGCACACGGAGCTATGTTATTTTATCTTTTGAAAACAT
GGTGACTACATGGACATGAAGCAGGCTGATACTACACAGTATGTC
CCCATGCTAGAAAGGAAAGAGGTTTCTAAATATTCCGACATCCAG
AGATCACTCTATGATCGTCCAGCCTCATATAAGAAGAAATCTATG
TTAGACTCAGAAGTCAAAAACCTCCTTTCAGATGATAACTCAGAA
GGCCTTACTTTATTGGATTTGTTGAGCTTCACCTATCAAGTTGCC
CGAGGAATGGAGTTTTTGGCTTCAAAAAATTGTGTCCACCGTGAT
CTGGCTGCTCGCAACGTCCTCCTGGCACAAGGAAAAATTGTGAAG
ATCTGTGACTTTGGCCTGGCCAGAGACATCATGCATGATTCGAAC
TATGTGTGCGAAAGGCAGTACCTTCTGCCCCGTGAAGTGGATGGCT
CCTGAGAGCATCTTTGACAACCTCTACACCACACTGAGTGATGTC
TGGTCTTATGGCATTCTGCTCTGGGAGATCTTTTCCCTTGGTGGC
ACCCCTTACCCCGGCATGATGGTGGATTCTACTTTCTACAATAAG
ATCAAGAGTGGGTACCGGATGGCCAAGCCTGACCACGCTACCAGT
GAAGTCTACGAGATCATGGTGAAATGCTGGAACAGTGAGCCGGAG
AAGAGACCTCCTTTTACCACCTGAGTGAGATTGTGGAGAATCTG
CTGCCTGGACAATATAAAAAGAGTTATGAAAAAATTCACCTGGAC
TTCCTGAAGAGTGACCATCCTGCTGTGGCACGCATGCGTGTGGAC
TCAGACAATGCATACATTGGTGTACCTACAAAAACGAGGAAGAC
AAGCTGAAGGACTGGGAGGGTGGTCTGGATGAGCAGAGACTGAGC
GCTGACAGTGGCTACATCATTCCTCTGCCTGACATTGACCCTGTC
CCTGAGGAGGAGGACCTGGGCAAGAGGAACAGACACAGCTCGCAG
ACCTCTGAAGAGAGTGCCATTGAGACGGGTTCCAGCAGTTCCACC
TTCATCAAGAGAGAGGACGAGACCATTGAAGACATCGACATGATG
GACGACATCGGCATAGACTCTTCAGACCTGGTGGAAAGACAGCTTC
CTGtaagcggccgc