

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of active Platelet-derived growth factor receptor, beta polypeptide [557 – 1106]**

<b><u>Enzyme description:-</u></b>	PDGFRB [557 - 1106]
<b><u>Clone number:-</u></b>	DU 63238
<b><u>Source:-</u></b>	Recombinant
<b><u>Expression system:-</u></b>	Baculovirus expression vector system
<b><u>Tag:-</u></b>	N-terminal GST
<b><u>Purification method:-</u></b>	GSH Sepharose

#### **Calculated molecular mass:-**

Monoisotopic           88, 846.30 daltons  
Average Mass           88, 903.46 daltons  
[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-**                   5.08

**Purity:-**                             >80 %

**Activation protocol:-**             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.

**Storage temperature:-**           -70 °C

#### **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**

**PDGFRB [557 - 1106]**

**Protein** PDGFRB [557 - 1106]

**Clone number** DU 63238

**Species** Human

**Accession number** NM\_002609.3

**Tags** N-terminal GST

**Baculovirus expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNK  
KFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKE  
RAEISMLEGAVLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKM  
FEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKL  
VCFKKRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSD  
LEVLFQGPLGSPEFQKKPRYEIRWKVIESVSSDGHEYIYVDPMQ  
**LPYDSTWELPRDQLVLRGRTLGS GAFGQVVEATAHGLSHSQATMK**  
**VAVKMLKSTARSSSEKQALMSELKIMSHLGPHLNVNLLGACTKG**  
**GPIYIITEYCRYGDLVDYLHRNKHTFLQHHS DKRRPPSAELYSN**  
**ALPVGLPLPSHVSLTGESDGGYMDMSKDESVDYVPMLDMKGDVK**  
**YADIESSNYMAPYDNYVPSAPERTCRATLINESPVLSYMDLVGF**  
**SYQVANGMEFLASKNCVHRDLAARNVLI CEGKLVKICDFGLARD**  
**IMRDSNYISKGSTFLPLKWMAPESIFNSLYTTLSDVWSFGILLW**  
**EIFTLGGTPYPELPMNEQFYNAIKRGYRMAQPAHASDEIYEIMQ**  
**KCWEKFEIRPPFSQLVLLLERLLGEGYKKKYQQVDEEFLRSDH**  
**PAILRSQARLPGFHGLRSPLDTSSVLYTAVQPNEGDNDYIIPLP**  
**DPKPEVADEGPLEGSPSLASSTLNEVNTSSTISCDSPLEPQDEP**  
**EPEPQLELQVEPEPELEQLPDSGCPAPRAEAEDSFL**

**Native sequence** Amino acids Q557 – L1106 (end residue) of human PDGFRB. Residue Q235 of the fusion protein is equivalent to Q557 of the native enzyme. The GST tag is located at residues 1 - 220.

**Protease cleavage** PreScission (LEVLFQGP) residues 221 - 228

**Cloning sites** *EcoR*1 and *Not*1 sites of pFastBac Dual.

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**Nucleotide  
sequence of insert**

gaattcCAGAAGAAGCCACGTTACGAGATCCGATGGAAGGTGATT  
GAGTCTGTGAGCTCTGACGGCCATGAGTACATCTACGTGGACCCC  
ATGCAGCTGCCCTATGACTCCACGTGGGAGCTGCCGCGGGACCAG  
CTTGTGCTGGGACGCACCCCTCGGCTCTGGGGCCTTTGGGCAGGTG  
GTGGAGGCCACGGCTCATGGCCTGAGCCATTCTCAGGCCACGATG  
AAAGTGGCCGTCAAGATGCTTAAATCCACAGCCCAGCAGTGAAG  
AAGCAAGCCCTTATGTTCGGAGCTGAAGATCATGAGTCACCTTGGG  
CCCCACCTGAACGTGGTCAACCTGTTGGGGGCCTGCACCAAAGGA  
GGACCCATCTATATCATCACTGAGTACTGCCGCTACGGAGACCTG  
GTGGACTACCTGCACCGCAACAAACACACCTTCCTGCAGCACCAC  
TCCGACAAGCGCCGCCCGCCAGCGCGGAGCTCTACAGCAATGCT  
CTGCCCCGTTGGGCTCCCCCTGCCAGCCATGTGTCCTTGACCGGG  
GAGAGCGACGGTGGCTACATGGACATGAGCAAGGACGAGTCGGTG  
GACTATGTGCCCATGCTGGACATGAAAGGAGACGTCAAATATGCA  
GACATCGAGTCCTCCAACACTACATGGCCCCTTACGATAACTACGTT  
CCCTCTGCCCTGAGAGGACCTGCCGAGCAACTTTGATCAACGAG  
TCTCCAGTGCTAAGCTACATGGACCTCGTGGGCTTCAGCTACCAG  
GTGGCCAATGGCATGGAGTTTCTGGCCTCCAAGAAGTGCCTCCAC  
AGAGACCTGGCGGCTAGGAACGTGCTCATCTGTGAAGGCAAGCTG  
GTCAAGATCTGTGACTTTGGCCTGGCTCGAGACATCATGCGGGAC  
TCGAATTACATCTCAAAGGCAGCACCTTTTTGCCTTTAAAGTGG  
ATGGCTCCGGAGAGCATCTTCAACAGCCTCTACACCACCCTGAGC  
GACGTGTGGTCTTTCGGGATCCTGCTCTGGGAGATCTTCACCTTG  
GGTGGCACCCCTTACCCAGAGCTGCCCATGAACGAGCAGTTCTAC  
AATGCCATCAAACGGGGTTACCGCATGGCCCAGCCTGCCATGCC  
TCCGACGAGATCTATGAGATCATGCAGAAGTGCTGGGAAGAGAAG  
TTTGAGATTTCGGCCCCCTTCTCCAGCTGGTGTGCTTCTCGAG  
AGACTGTTGGGCGAAGGTTACAAAAAGAAGTACCAGCAGGTGGAT  
GAGGAGTTTCTGAGGAGTGACCACCCAGCCATCCTTCGGTCCCAG  
GCCCCGCTTGCCTGGGTTCCATGGCCTCCGATCTCCCCTGGACACC  
AGCTCCGTCCTCTATACTGCCGTGCAGCCCAATGAGGGTGACAAC  
GACTATATCATCCCCCTGCCTGACCCCAAACCCGAGGTTGCTGAC  
GAGGGCCCACTGGAGGGTTCCCCCAGCCTAGCCAGCTCCACCCTG  
AATGAAGTCAACACCTCCTCAACCATCTCCTGTGACAGCCCCCTG  
GAGCCCCAGGACGAACCAGAGCCAGAGCCCCAGCTTGAGCTCCAG  
GTGGAGCCGGAGCCAGAGCTGGAACAGTTGCCGGATTTCGGGGTGC  
CCTGCGCCTCGGGCGGAAGCAGAGGATAGCTTCCTGtaggcggcc  
gc