

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

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

<b><u>Enzyme description:-</u></b>	PDGFRA [551 – 10589]
<b><u>Clone number:-</u></b>	DU 4793
<b><u>Source:-</u></b>	Recombinant
<b><u>Expression system:-</u></b>	Baculovirus expression vector system
<b><u>Tag:-</u></b>	N-terminal His(6) tag
<b><u>Purification method:-</u></b>	Ni <sup>2+</sup> -NTA agarose

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

Monoisotopic        64, 759.90 daltons  
Average Mass        64, 801.18 daltons  
[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-**                      5.27

**Purity:-**                                      80 %

#### **Activation protocol:-**

Autoactivated by incubation with Mn / ATP followed by dialysis to remove excess ATP

#### **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 °C

#### **Assay buffer:-**

50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 2 mM MnCl<sub>2</sub>

#### **Substrate:-**

KKKKEEIYFFFG                      Final concentration: 300 μM

*Division of Signal Transduction Therapy*

**Clone Data Sheet**

**PDGFRA [551 – 1089]**

**Protein** PDGFRA [551 - 1089]

**Clone number** DU 4793

**Species** Human

**Accession number** NM\_006206

**Tags** N-terminal His(6)

**Baculovirus  
expressed protein**

MSY~~Y~~HHHHHHHDYDIPTT~~ENLYFQ~~GAMGSQKPRYEIRWRVIESISP  
DGHEYIYVDPMQLPYDSRWEFPRDGLVLGRVLGSGAFGKVVETA  
YGLSRSQPVMKVAVKMLKPTARSSEKQALMSELKIMTHLGPHLNI  
VNLGACTKSGPIYIITEYCFYGDLVNYLHKNRDSFLSHHPEKPK  
KELDIFGLNPADESTRSYVILSFENNGDYMDMKQADTTQYVPMLE  
RKEVSKYSYDIQRSYDRPASYKKKSMLDSEVKNLLSDDNSEGLTL  
LDLLSFTYQVARGMEFLASKNCVHRDLAARNVLLAQQKIVKICDF  
GLARDIMHDSNYVSKGSTFLPVKWMAPESIFDNLYTTLSDVWSYG  
ILLWEIFSLGGTPYPGMMVDSTFYNKIKSGYRMAKPDHATSEVYE  
IMVKCWNSEPEKRPSFYHLSEIVENLLPGQYKKSYEKIHLDLFLKS  
DHPAVARMRVSDNAYIGVTYKNEEDKLDWEGGLDEQRLSADSG  
YIIPDPDIDPVPEEEDLGKRNRRHSSQTSEESALETGSSSSTFIKR  
EDETIEDIDMMDDIGIDSSDLVEDSFL

**Native sequence** Amino acids Q551 – L1089 (end) of human PDGFRA.  
Residue Q29 of the fusion protein is equivalent to Q551 of the  
native enzyme. The His(6) tag is located at residues 5 - 10.

**Protease cleavage** rTEV (ENLYFQG) residues 18 - 24

**Cloning sites** *Bam*H1 and *Not*I sites of pFBHTb

*Division of Signal Transduction Therapy*

**Complete  
Nucleotide  
sequence**

ATGTCGTA CTACCATC ACCATC ACCATC AC GATTAC GATATCCCA  
ACGACCGAAA CCTGTATTTT CAGGGCGCCATGGGATCCCAGAAA  
CCGAGGTATGAAATTCGCTGGAGGGTCATTGAATCAATCAGCCC  
GATGGACATGAATATATTTATGTGGACCCGATGCAGCTGCCTTAT  
GACTCAAGATGGGAGTTTCCAAGAGATGGACTAGTGCTTGGTCGG  
GTCTTGGGGTCTGGAGCGTTTGGGAAGGTGGTTGAAGGAACAGCC  
TATGGATTAAGCCGGTCCCAACCTGTCATGAAAGTTGCAGTGAAG  
ATGCTAAAACCCACGGCCAGATCCAGTGAAAAACAAGCTCTCATG  
TCTGAACTGAAGATAATGACTCACCTGGGGCCACATTTGAACATT  
GTAAACTTGCTGGGAGCCTGCACCAAGTCAGGCCCCATTTACATC  
ATCACAGAGTATTGCTTCTATGGAGATTTGGTCAACTATTTGCAT  
AAGAATAGGGATAGCTTCCCTGAGCCACCACCCAGAGAAGCCAAAG  
AAAGAGCTGGATATCTTTGGATTGAACCTGCTGATGAAAGCACA  
CGGAGCTATGTTATTTTATCTTTTGA AAAACAATGGTGACTACATG  
GACATGAAGCAGGCTGATACTACACAGTATGTCCCATGCTAGAA  
AGGAAAGAGGTTTCTAAATATTCCGACATCCAGAGATCACTCTAT  
GATCGTCCAGCCTCATATAAGAAGAAATCTATGTTAGACTCAGAA  
GTCAAAAACCTCCTTTT CAGATGATAACTCAGAAGGCCTTACTTTA  
TTGGATTTGTTGAGCTTCACCTATCAAGTTGCCCGAGGAATGGAG  
TTTTTGGCTTCAAAA AATTGTGTCCACCGTGATCTGGCTGCTCGC  
AACGTCTCCTGGCACAAGGAAAAATTGTGAAGATCTGTGACTTT  
GGCCTGGCCAGAGACATCATGCATGATTCGAACTATGTGTCGAAA  
GGCAGTACC'TTTCTGCCCGTGAAGTGGATGGCTCCTGAGAGCATC  
TTTGACAACCTCTACACCACACTGAGTGATGTCTGGTCTTATGGC  
ATTCTGCTCTGGGAGATCTTTTCCCTTGGTGGCACCCCTTACCC  
GGCATGATGGTGGATTCTACTTTCTACAATAAGATCAAGAGTGGG  
TACCGGATGGCCAAGCCTGACCACGCTACCAGTGAAGTCTACGAG  
ATCATGGTGAAATGCTGGAACAGTGAGCCGGAGAAGAGACCCTCC  
TTTTACCACCTGAGTGAGATTGTGGAGAATCTGCTGCCTGGACAA  
TATAAAAAGAGTTATGAAAAAATTCACCTGGACTTCTGAAGAGT  
GACCATCCTGCTGTGGCACGCATGCGTGTGGACTCAGACAATGCA  
TACATTGGTGTACCTACAAAAACGAGGAAGACAAGCTGAAGGAC  
TGGGAGGGTGGTCTGGATGAGCAGAGACTGAGCGCTGACAGTGGC  
TACATCATTCCTCTGCCTGACATTGACCCTGTCCCTGAGGAGGAG  
GACCTGGGCAAGAGGAACAGACACAGCTCGCAGACCTCTGAAGAG  
AGTGCCATTGAGACGGGTTCCAGCAGTTCACCTTCATCAAGAGA  
GAGGACGAGACCATTGAAGACATCGACATGATGGACGACATCGGC  
ATAGACTCTTCAGACCTGGTGGAAAGACAGCTTCCCTGtaagcggcc  
gc