

Certificate of Analysis

PDGFR beta, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-463, 14-463-K, 14-463M

Parent Lot # 2210531

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal 6His-tagged recombinant human PDGFR beta residues 557–end. Expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 87.3% by SDS-PAGE and Coomassie blue staining. MW = 64kDa.

Specific Activity (Parent lot# 2210531): 64U/mg, where one unit of PDGFR beta, active activity is defined as 1nmol phosphate incorporated into poly(Glu, Tyr) (4:1) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.975mg/ml of enzyme in 50mM Tris/HCl pH7.5, 0.1mM EGTA, 150mM NaCl, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

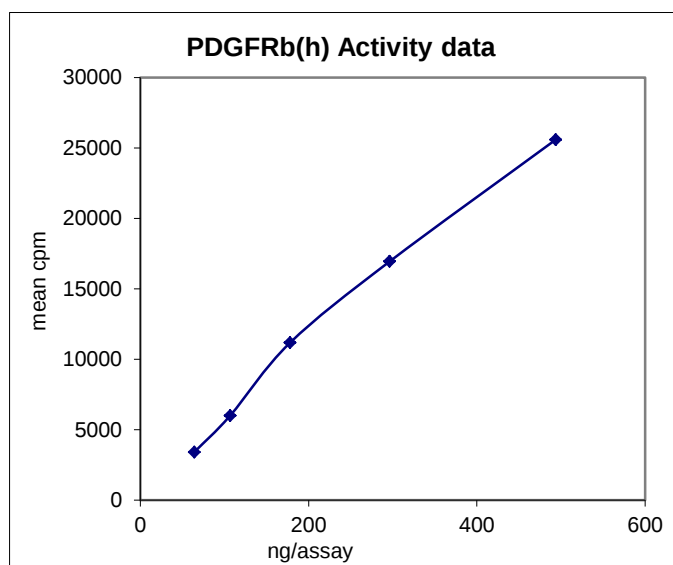
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

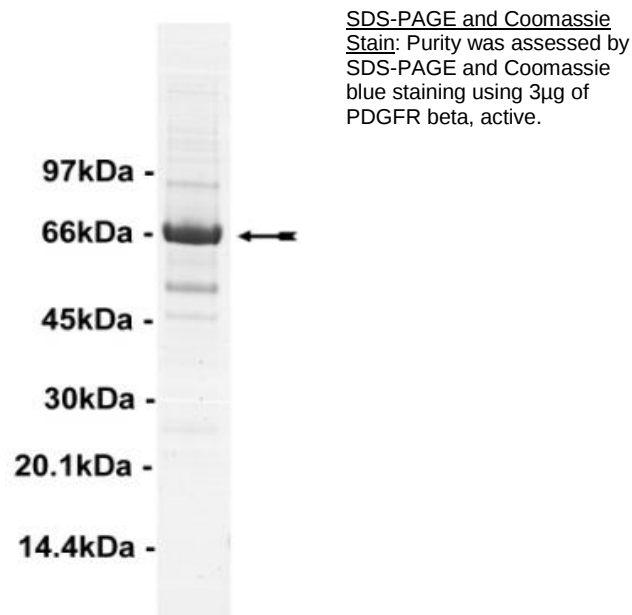
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 64–494ng of this lot of enzyme phosphorylated 0.1mg/ml poly(Glu, Tyr) (4:1) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed product identity as PDGFR beta with the translated sequence listed on page three.



Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **MnCl₂:** Use at a final assay concentration of 10mM. Prepare a 200mM stock in dH₂O and add 1.25µl of stock per assay point.
3. **Poly(Glu, Tyr) (4:1):** Use at a final assay concentration of 0.1mg/ml. Prepare a 1mg/ml stock and add 2.5µl of stock per assay point.
4. **PDGFR beta, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 64–494ng per assay point.
5. **[γ-³³P]ATP:** 2.5 x magnesium acetate/[γ-³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **poly(Glu, Tyr) (4:1)**.
3. Add 1.25µl of MnCl₂.
4. Add **2.5µl (64–494ng) PDGFR beta, active**.
5. Add 3.75µl of dH₂O.
6. Add 10µl of diluted [γ-³³P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
9. Transfer a 10µl aliquot onto the appropriate area of a **Filtermat A**.
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

Certificate of Analysis

PDGFR beta Sequence Information

<u>Protein</u>	human PDGFR beta
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	Q16 of the recombinant protein is equivalent to Q557 of human PDGFR beta
<u>Accession number</u>	GenBank J03278

Recombinant PDGFR beta amino acid sequence:

```

1 MAHHHHHHEN LYFQGQKKPR YEIRWKVIES VSSDGHEYIY VDPMQLPYDS TWELPRDQLV
61 LGRTLGSAGF GQVVEATAHG LSHSQATMKV AVKMLKSTAR SSEKQALMSE LKIMSHLGPH
121 LNVNLLGAC TKGGPIYIIT EYCRYGDLVD YLHRNKHTFL QHHS DKRRPP SAELYSNALP
181 VGLPLPSHVS LTGESDGGYM DMSKDESVDY VPMLDMKGDV KYADIESSNY MAPYDNYVPS
241 APERTCRATL INESPVLSYM DLVGFSYQVA NGMEFLASKN CVHRDLAARN VLICEGKLVK
301 ICDFLGARDI MRDSNYISKG STFLPLKUMA PESIFNSLYT TLDVWSFGI LLWEIFTLGG
361 TPYPELPMNE QFYNAIKRGY RMAQPAHASD EIYEIMQKCW EEKFEIRPPF SQLVLLLERL
421 LGEYKKKYQ QVDEEFLRSD HPAILRSQAR LPGFHGLRSP LDTSSVLYTA VQPNEGDNDY
481 IIPLPDPKPE VADEGPLEGS PSLASSTLNE VNTSSTISCD SPLEPQDEPE PEPQLELQVE
541 PEPELEQLPD SGCPAPRAEA EDSFL

```

Recombinant PDGFR beta nucleotide sequence:

```

1 atggcgcatc accatcacca tcatgaaaac ctgtattttc agggccagaa gaagccacgt
61 tacgagatcc gatggaaggt gattgagtct gtgagctctg acggccatga gtacatctac
121 gtggacccca tgcagctgcc ctatgactcc acgtgggagc tgccgcggga ccagcttggtg
181 ctgggacgca cctcggctc tggggccttt gggcaggtgg tggaggccac ggctcatggc
241 ctgagccatt ctcaggccac gatgaaagt gccgtcaaga tgcttaaata cacagcccgc
301 agcagtgaga tgaagccct tatgtcggag ctgaagatca tgagtcacct tgggccccac
361 ctgaacgtgg tcaacctgtt gggggcctgc accaaaggag gaccatcta tatcatcact
421 gagtactgcc gctacggaga cctggtggac tacctgcacc gcaacaaaca caccttcctg
481 cagcaccact ccgacaagcg ccgcccggcc agcgcggagc tctacagcaa tgctctgccc
541 gttgggctcc cctgcccag ccatgtgtcc ttgaccgggg agagcgacgg tggctacatg
601 gacatgagca aggacgagtc ggtggactat gtgccatgc tggacatgaa aggagacgtc
661 aaatatgcag acatcgagtc ctccaactac atggcccctt acgataacta cgttcctctt
721 gccctgaga ggacctgcc agcaactttg atcaacgagt ctccagtgtc aagctacatg
781 gacctcgtgg gcttcagcta ccaggtggcc aatggcatgg agtttctggc ctccaagaac
841 tgcgtccaca gagacctggc ggctaggaac gtgctcatct gtgaaggcaa gctggtcaag
901 atctgtgact ttggcctggc tcgagacatc atgcgggact cgaattacat ctccaaaggc
961 agcacctttt tgcctttaa gtggatggct ccggagagca tcttcaacag cctctacacc
1021 accctgagcg acgtgtggtc cttcgggatc ctgctctggg agatcttcac cttgggtggc
1081 accccttacc cagagctgcc catgaacgag cagttctaca atgccatcaa acgggggttac
1141 cgcattggcc agcctgccc tgcctccgac gagatctatg agatcatgca gaagtgtctg
1201 gaagagaagt ttgagattcg gcccccttc tcccagctgg tgctgcttct cgagagactg
1261 ttgggcgaag gttacaaaaa gaagtaccag caggtggatg aggagtttct gaggagtga
1321 caccagcca tccttcggtc ccaggccgc ttgcctgggt tccatggcct ccgatctccc
1381 ctggacacca gctccgtcct ctatactgcc gtgcagccca atgagggtga caacgactat
1441 atcatcccc tgcctgacct caaacccgag gttgctgacg agggccact ggagggttcc
1501 cccagcctag ccagctccac cctgaatgaa gtcaaacact cctcaaccat ctcctgtgac

```

Certificate of Analysis

1561 agccccctgg agccccagga cgaaccagag ccagagcccc agcttgagct ccaggtggag
1621 ccggagccag agctggaaca gttgccggat tcggggtgcc ctgcgcctcg ggcggaagca
1681 gaggatagct tcctgtag

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 **Eurofins Pharma Discovery Services UK Limited** is an independent member of Eurofins Discovery Services