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Certificate of Analysis

PDGFR alpha (550-end), active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-467, 14-467-K, 14-467M
Parent Lot # 1767085

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 alpha residues 550–end. Expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose. Purity 80.3% by SDS-PAGE and Coomassie blue staining. MW = 63.5kDa.

Specific Activity (Parent lot# 1767085): 29U/mg, where one unit of PDGFR alpha, active activity is defined as 1nmol phosphate incorporated into 0.1mg/ml poly(Glu, Tyr) (4:1) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.127mg/ml of enzyme in 50mM Tris/HCl pH7.5, 0.1mM EGTA, 150mM NaCl, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

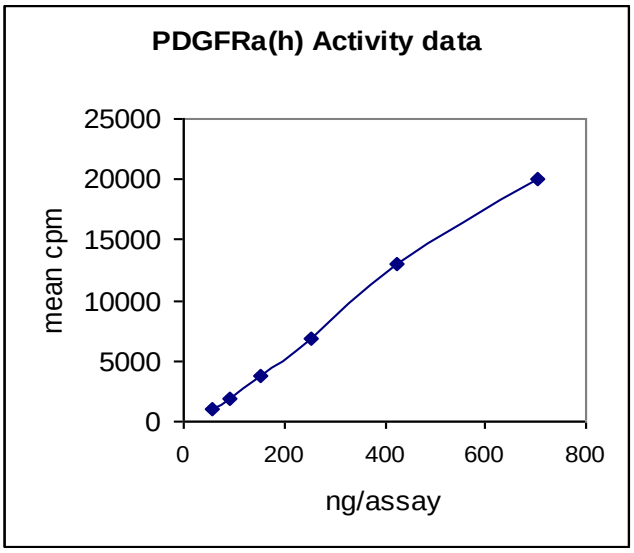
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

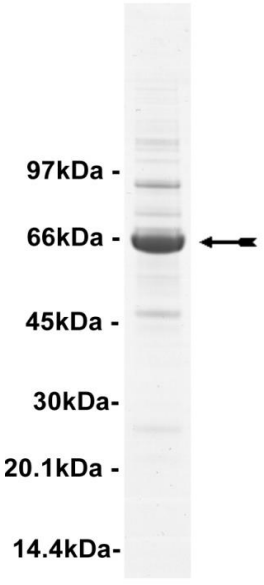
Quality Control Testing

Kinase Assay: 55–704ng of this lot of enzyme phosphorylated 0.1mg/ml poly(Glu, Tyr) 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 alpha with the translated sequence listed on page three.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of PDGFR alpha, active.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **$MnCl_2$:** Use at a final assay concentration of 10mM. Prepare a 200mM stock in dH_2O 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 alpha, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1 % 2-mercaptoethanol, 1mg/ml BSA. Use 55–704ng per assay point.
5. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]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 $MnCl_2$.
4. Add **2.5µl (55–704ng) PDGFR alpha, active**.
5. Add 3.75µl of dH_2O.
6. Add 10µl of diluted [γ-^{33}P]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.

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PDGFR alpha Sequence Information

Protein human PDGFR alpha

Tags *N*-terminal 6His

Native sequence K16 of the recombinant protein is equivalent to K550 of human PDGFR alpha

Accession number GenBank M21574

Recombinant PDGFR alpha amino acid sequence:

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   1 MAHHHHHHEN LYFQGKQKPR YEIRWRVIES ISPDGHEYIY VDPMQLPYDS RWEFPRDGLV
  61 LGRVLGSGAF GKVVEGTAYG LSRSQPVMKV AVKMLKPTAR SSEKQALMSE LKIMTHLGPH
 121 LNIVNLLGAC TKSGPIYIIT EYCFYGDLVN YLHKNRDSFL SHHPEKPKKE LDIFGLNPAD
 181 ESTRSYVILS FENNGDYMDM KQADTTQYVP MLERKEVSKY SDIQRSLYDR PASYKKKSML
 241 DSEVKNLLSD DNSEGLTLLD LLSFTYQVAR GMEFLASKNC VHRDLAARNV LLAQGKIVKI
 301 CDFGLARDIM HDSNYVSKGS TFLPVKWMAP ESIFDNLYTT LSDVWSYGIL LWEIFSLGGT
 361 PYPGMMVDST FYNKIKSGYR MAKPDHATSE VYEIMVKCWN SEPEKRPSFY HLSEIVENLL
 421 PGQYKKSYEK IHLDFLKSDH PAVARMRVDS DNAYIGVTYK NEEDKLKDWE GGLDEQRLSA
 481 DSGYIIPLPD IDPVPEEEDL GKRNRHSSQT SEESAIETGS SSSTFIKRED ETIEDIDMMD
 541 DIGIDSSDLV EDSFL
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Recombinant PDGFR alpha nucleotide sequence:

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   1 atggcgcatc accatcacca tcatgaaaac ctgtattttc agggcaaaca gaaaccgagg
  61 tatgaaattc gctggagggt cattgaatca atcagcccgg atggacatga atatatttat
 121 gtggacccga tgcagctgcc ttatgactca agatgggagt ttccaagaga tggactagtg
 181 cttggtcggg tcttggggtc tggagcgttt gggaaggtgg ttgaaggaac agcctatgga
 241 ttaagccggt cccaacctgt catgaaagtt gcagtgaaga tgctaaaacc cacggccaga
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 541 gaaagcacac ggagctatgt tattttatct tttgaaaaca atggtgacta catggacatg
 601 aagcaggctg atactacaca gtatgtcccc atgctagaaa ggaaagaggt ttctaaatat
 661 tccgacatcc agagatcact ctatgatcgt ccagcctcat ataagaagaa atctatgtta
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1621 gacatcggca tagactcttc agacctggtg gaagacagct tcctgtaa
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Reviewed and approved by site quality representative.