

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 Ni²⁺/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.

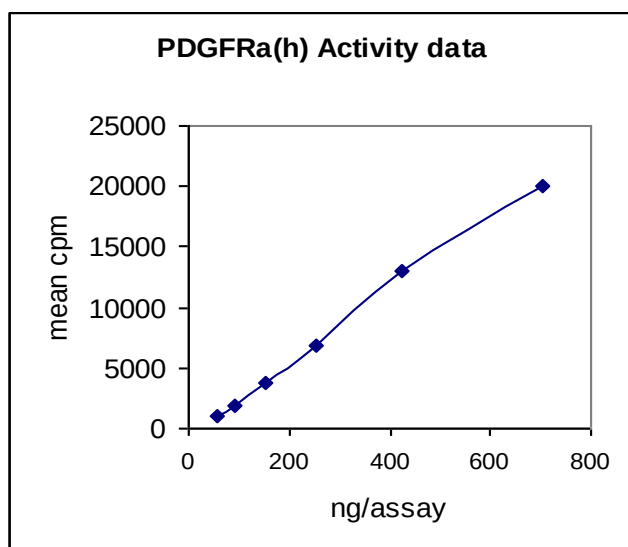
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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**

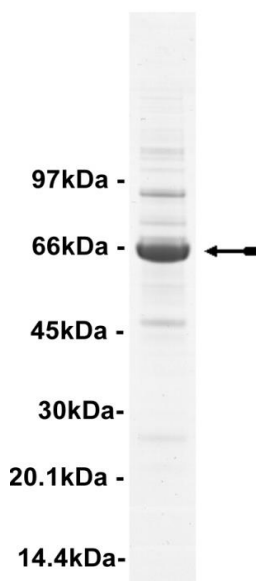
Quality Control Testing

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. **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 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-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 (55–704ng) PDGFR alpha, 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.

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 LYFQKQKPR YEIRWRVIES ISPDGHEYIY VDPMQLPYDS RWEFPRDGLV
61 LGRVLGSGAF GKVEGTAYG LRSQPVMMKV AVKMLKPTAR SSEKQALMSE LKIMTHLGPH
121 LNIVNLLGAC TKS GPIYIIT EYCFYGD LVN 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 PGQYKKSIEK IHLDFLKSDH PAVARMRVDS DNAYIGVTYK NEEDKLDWE GGLDEQRLSA
481 DSGYIIPLPD IDPVPEEEDL GKRNRHSSQT SEESAIE TGS 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 gtggaccgca tgcagctgcc ttatgactca agatgggagt ttccaagaga tggactagtg
181 cttggctcggg tcttgggggtc tggagcggtt gggaagggtg ttgaaggaa acgctatgga
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1621 gacatcgga tagactcttc agacctggtg gaagacagct tcctgtaa

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