

Certificate of Analysis

PDGFR alpha (550–end, D842V), active (Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-729, 14-729-K, 14-729M

Parent Lot # 609040604

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 amino acids 550–end, containing the D842V mutation. Expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose.

This D842V mutation has been found in patients with gastrointestinal stromal tumours (GIST) and is identical to that of c-Kit D816V. This mutation results in the partial inhibition of PDGFR alpha by Gleevec® (Hirota S *et al.*, Gastroenterology, (2003):125;660-667.)

Purity 68% by SDS-PAGE and Coomassie blue staining. MW = 63.5kDa.

Formulation: 2.444mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 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.

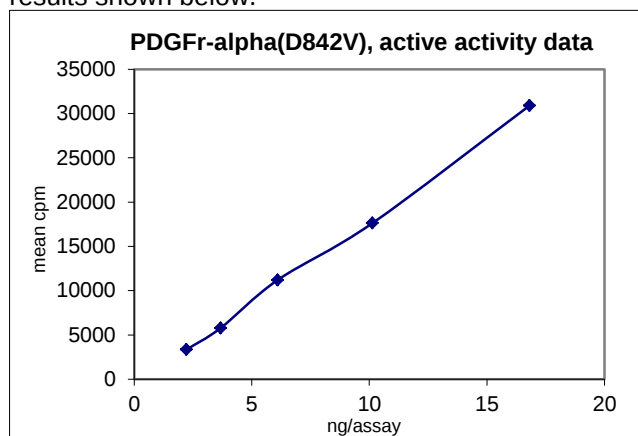
Specific Activity (Parent lot# 609040604): 858U/mg, where one unit of PDGFR alpha (D842V), active activity is defined as 1nmol phosphate incorporated into 250µM (GGMEDYFEFMGGKKK) per minute at 30°C with a final ATP concentration of 100µM.

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.

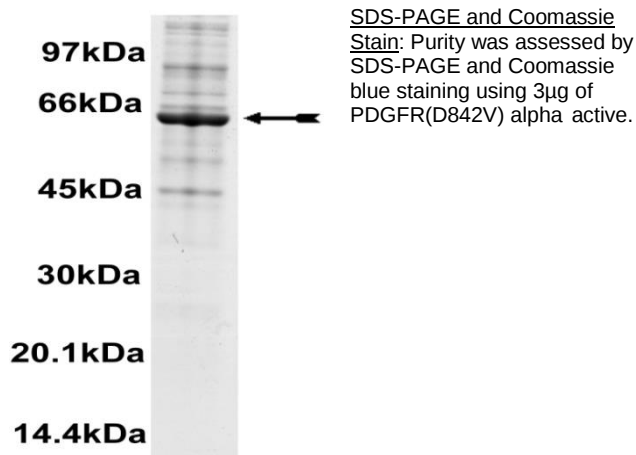
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 2–17ng of this lot of enzyme phosphorylated 250µM (GGMEDYFEFMGGKKK) 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 identity as PDGFR alpha (D842V) with the translated native sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **(GGMEDIYFEFMGGKKK):** Use at a final assay concentration of 250µM. Make up a 2.5mM stock. Add 2.5µl of stock per assay point.
3. **PDGFR alpha (D842V), active:** dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 2-17ng per assay point.
4. **[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 (GGMEDIYFEFMGGKKK).
3. Add **2.5µl (2–17ng) PDGFR alpha (D842V), active.**
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. 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 (D842V) sequence Information

Protein	Human PDGFR alpha (D842V)
Tags	N-terminal 6His
Native sequence	K16 of recombinant sequence is equivalent to K550 of native human PDGFR alpha
Accession number	Genbank M21574

Recombinant PDGFR alpha (D842V) 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 EYCFYGLVN YLHKNRDSFL SHHPEKPKKE LDIFGLNPAD
181 ESTRSYVILS FENNGDYMDM KQADTTQYVP MLERKEVSKY SDIQRSLYDR PASYKKKSML
241 DSEVKNLLSD DNSEGLTLLD LLSFTYQVAR GMEFLASKNC VHRDLAARNV LLAQGKIVKI
301 CDFGLARVIM HDSNYVSKGS TFLPVKWMAP ESIFDNLTYT LSDVWSYGIL LWEIFSLGGT
361 PYPGMMVDST FYNKIKSGYR MAKPDHATSE VYEIMVKCWN SEPEKRPSFY HLSEIVENLL
421 PGQYKKSIEK IHLDFLKSDH PAVARMRVDS DNAYIGVTYK NEEDKLKDWE GGLDEQRLSA
481 DSGYIIPLPD IDPVPEEEDL GKRNRHSSQT SEESAIETGS SSSTFIKRED ETIEDIDMMD
541 DIGIDSSDLV EDSFL

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Recombinant PDGFR alpha (D842V) nucleotide sequence:

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1 atggcgcatc accatcacca tcatgaaac ctgtattttc agggcaaaca gaaaccgagg
61 tatgaaattc gctggagggt cattgaatca atcagcccgg atggacatga atatatttat
121 gtggacccga tgcagctgcc ttatgactca agatgggagt ttccaagaga tggactagtg
181 ctggctcggg tcttgggggtc tggagcggtt gggaagggtg ttgaaggaa acgctatgga
241 ttaagccggt cccaacctgt catgaaagt gcagtgaaga tgctaaaacc caggccaga
301 tccagtga aaacaagctct catgtctgaa ctgaagataa tgactcacct ggggccacat
361 ttgaacattg taaacttgct gggagcctgc accaagtcag gccccattta catcatcaca
421 gagtattgct tctatggaga tttgtcaac tatttgcata agaataggga tagcttcctg
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601 aagcaggctg atactacaca gtatgtcccc atgctagaaa ggaaagagg ttctaataat
661 tccgacatcc agagatcact ctatgctcgt ccagcctcat ataagaagaa atctatgtta
721 gactcagaag tcaaaaacct cctttcagat gataactcag aaggccttac tttattggat
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1501 ggcaagagga acagacacag ctgcagacc tctgaagaga gtgccattga gacgggttcc
1561 agcagttcca ccttcatcaa gagagaggac gagaccattg aagacatcga catgatggac
1621 gacatcgga tagactcttc agacctggtg gaagacagct tcctgtaa

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Reviewed and approved by site quality representative.

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