Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ PDGFR-alpha D842V

platelet derived growth factor receptor alpha

Recombinant Human Active Protein Kinase

HGNC Symbol: PDGFRA

Synonyms: PDGFR2, CD140a, GAS9

Product No.: 0761-0000-1

Lot: 002

Description: Human PDGFR-alpha, C-terminal fragment, amino acids Q_{551}-L_{1089} (as in NCBI/Protein entry NP_006197.1) with a D842V mutation, N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: PDGFR-alpha D842V Lot 002, was confirmed as PDGFR-alpha D842V by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 89,877 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

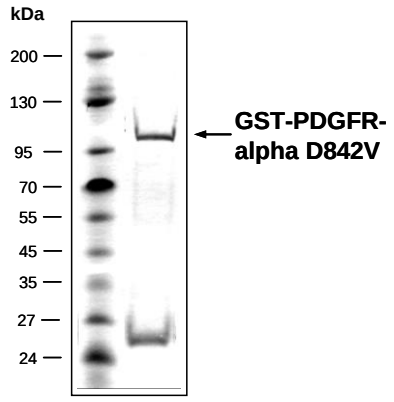
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

Storage temperature: -80°C
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.064 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

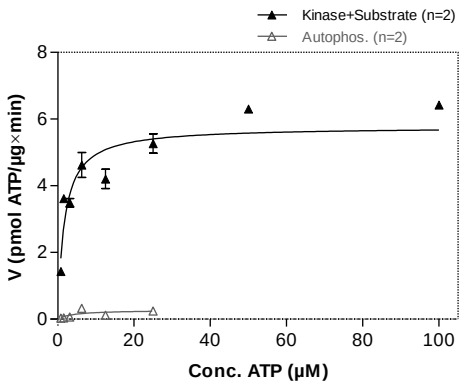
Biochemical Parameters:
Specific kinase activity (P_i transfer): 6 pmol/µg × min
ATP-K_M: 1.7 µM

PDGFR-alpha D842V Lot 002: Coomassie stain



2.0 µg GST-PDGFR-alpha D842V

PDGFR-alpha D842V Lot 002: Determination of V_{max} and K_M value for ATP



- Assay conditions:
  60 mM HEPES-NaOH, pH 7.5
  3 mM $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg/ml $PEG_{20.000}$
  ATP (variable)
  Substrate: TRK-C-derived peptide 80 µg/ml
  Kinase: 1 µg/ml
- Filter binding assay
  MSPH membrane (Millipore)

Additional assay technology:
PDGFR-alpha D842V Lot 002 was also successfully tested by Reaction Biology for the use with the ADP-Glo™ Kinase assay from Promega ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

ProQinase™ PDGFR-alpha D842V

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GST-PDGFR-alpha D842V Recombinant Fusion Protein Amino Acid Sequence

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   1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID  60
  61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV 120
 121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
 181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RDSLEVLFQG 240
 241 PLAMGQKPRY EIRWRVIESI SPDGHEYIYV DPMQLPYDSR WEFPRDGLVL GRVLGSGAFG 300
 301 KVVEGTAYGL SRSQPVMKVA VKMLKPTARS SEKQALMSEL KIMTHLGPHL NIVNLLGACT 360
 361 KSGPIYIITE YCFYGDLVNY LHKNRDSFLS HHPEKPKKEL DIFGLNPADE STRSYVILSF 420
 421 ENNGDYMDMK QADTTQYVPM LERKEVSKYS DIQRSLYDRP ASYKKKSMLD SEVKNLLSDD 480
 481 NSEGLTLLDL LSFTYQVARG MEFLASKNCV HRDLAARNVL LAQGKIVKIC DFGLARVIMH 540
 541 DSNYVSKGST FLPVKWMAPE SIFDNLYTTL SDVWSYGILL WEIFSLGGTP YPGMMVDSTF 600
 600 YNKIKSGYRM AKPDHATSEV YEIMVKCWNS EPEKRPSFYH LSEIVENLLP GQYKKSYEKI 660
 661 HLDFLKSDHP AVARMRVDSD NAYIGVTYKN EEDKLKDWEG GLDEQRLSAD SGYIIPLPDI 720
 721 DPVPEEEDLG KRNRHSSQTS EESAIETGSS SSTFIKREDE TIEDIDMMDD IGIDSSDLVE 780
 781 DSFL                                                              840
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1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: PDGFR-alpha fragment **boxed**: D842V mutation

PDGFR-alpha wt[1] Amino Acid Sequence

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   1 MGTSHPAFLV LGCLLTGLSL ILCQLSLPSI LPNENEKVVQ LNSSFSLRCF GESEVSWQYP  60
  61 MSEEESSDVE IRNEENNSGL FVTVLEVSSA SAAHTGLYTC YYNHTQTEEN ELEGRHIYIY 120
 121 VPDPDVAFVP LGMTDYLVIV EDDDSAIIPC RTTDPETPVT LHNSEGVVPA SYDSRQGFNG 180
 181 TFTVGPYICE ATVKGKKFQT IPFNVYALKA TSELDLEMEA LKTVYKSGET IVVTCAVFNN 240
 241 EVVDLQWTYP GEVKGKGITM LEEIKVPSIK LVYTLTVPEA TVKDSGDYEC AARQATREVK 300
 301 EMKKVTISVH EKGFIEIKPT FSQLEAVNLH EVKHFVVEVR AYPPPRISWL KNNLTLIENL 360
 361 TEITTDVEKI QEIRYRSKLK LIRAKEEDSG HYTIVAQNED AVKSYTFELL TQVPSSILDL 420
 421 VDDHHGSTGG QTVRCTAEGT PLPDIEWMIC KDIKKCNNET SWTILANNVS NIITEIHSRD 480
 481 RSTVEGRVTF AKVEETIAVR CLAKNLLGAE NRELKLVAPT LRSELTVAAA VLVLLVIVII 540
 541 SLIVLVVIWK QKPRYEIRWR VIESISPDGH EYIYVDPMQL PYDSRWEFPR DGLVLGRVLG 600
 600 SGAFGKVVEG TAYGLSRSQP VMKVAVKMLK PTARSSEKQA LMSELKIMTH LGPHLNIVNL 660
 661 LGACTKSGPI YIITEYCFYG DLVNYLHKNR DSFLSHHPEK PKKELDIFGL NPADESTRSY 720
 721 VILSFENNGD YMDMKQADTT QYVPMLERKE VSKYSDIQRS LYDRPASYKK KSMLDSEVKN 780
 781 LLSDDNSEGL TLLDLLSFTY QVARGMEFLA SKNCVHRDLA ARNVLLAQGK IVKICDFGLA 840
 841 RDIMHDSNYV SKGSTFLPVK WMAPESIFDN LYTTLSDVWS YGILLWEIFS LGGTPYPGMM 900
 901 VDSTFYNKIK SGYRMAKPDH ATSEVYEIMV KCWNSEPEKR PSFYHLSEIV ENLLPGQYKK 960
 961 SYEKIHLDFL KSDHPAVARM RVDSDNAYIG VTYKNEEDKL KDWEGGLDEQ RLSADSGYII 1020
1021 PLPDIDPVPE EEDLGKRNRH SSQTSEESAI ETGSSSSTFI KREDETIEDI DMMDDIGIDS 1080
1081 SDLVEDSFL                                                         1140
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blue: PDGFR-alpha sequence expressed in recombinant protein **Red**: variant in recombinant protein

[1]NCBI/Protein accession number NP_006197.1

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