Certificate of Analysis

FAK aa2-1052

focal adhesion kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: PTK2

Synonyms: FADK, FADK1, FAK1, FRNK, pp125FAK

Product No.: 0165-0000-3

Lot: 016

Description: Human FAK, amino acids A_2-H_{1052} (as in NCBI/Protein entry NP_722560.1), activated, N-terminal GST fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: FAK Lot 016, was confirmed as FAK by by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 145,394 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: in-vitro activation by SRC

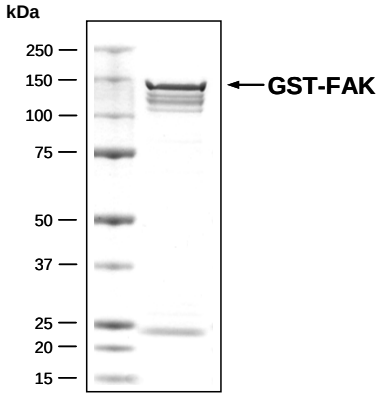
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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

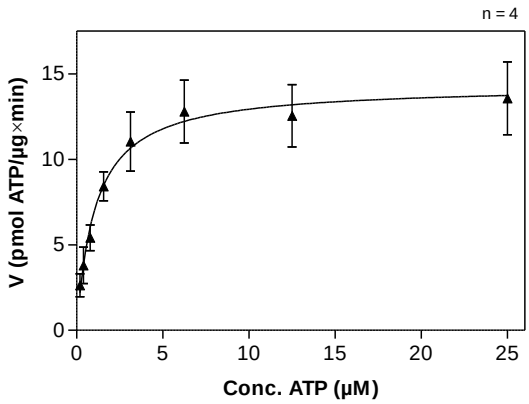
Biochemical Parameters:
Specific kinase activity (P_i transfer): 14 pmol/µg × min
ATP-K_M: 1.1 µM

FAK Lot 016:
Coomassie stain



2.0 µg GST-FAK

FAK Lot 016:
Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:
- 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: $Poly(Glu/Tyr)_{4:1}$ 5 µg/ml
  Kinase: 1 µg/ml
- Filter binding assay
  MSFC membrane (Millipore)

Additional assay technology: FAK Lot 016
was also successfully tested by ProQinase 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

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FAK

Product No.: 0165-0000-3

GST-FAK 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 LVPRGSAAAY LDPNLNHTPN 240
 241 SSTKTHLGTG MERSPGAMER VLKVFHYFES NSEPTTWASI IRHGDATDVR GIIQKIVDSH 300
 301 KVKHVACYGF RLSHLRSEEV HWLHVDMGVS SVREKYELAH PPEEWKYELR IRYLPKGFLN 360
 361 QFTEDKPTLN FFYQQVKSDY MLEIADQVDQ EIALKLGCLE IRRSYWEMRG NALEKKSNYE 420
 421 VLEKDVGLKR FFPKSLLDSV KAKTLRKLIQ QTFRQFANLN REESILKFFE ILSPVYRFDK 480
 481 ECFKCALGSS WIISVELAIG PEEGISYLTD KGCNPTHLAD FTQVQTIQYS NSEDKDRKGM 540
 541 LQLKIAGAPE PLTVTAPSLT IAENMADLID GYCRLVNGTS QSFIIRPQKE GERALPSIPK 600
 601 LANSEKQGMR THAVSVSETD DYAEIIDEED TYTMPSTRDY EIQRERIELG RCIGEGQFGD 660
 661 VHQGIYMSPE NPALAVAIKT CKNCTSDSVR EKFLQEALTM RQFDHPHIVK LIGVITENPV 720
 721 WIIMELCTLG ELRSFLQVRK YSLDLASLIL YAYQLSTALA YLESKRFVHR DIAARNVLVS 780
 781 SNDCVKLGDF GLSRYMEDST YYKASKGKLP IKWMAPESIN FRRFTSASDV WMFGVCMWEI 840
 841 LMHGVKPFQG VKNNDVIGRI ENGERLPMPP NCPPTLYSLM TKCWAYDPSR RPRFTELKAQ 900
 901 LSTILEEEKA QQEERMRMES RRQATVSWDS GGSDEAPPKP SRPGYPSPRS SEGFYPSPQH 960
 961 MVQTNHYQVS GYPGSHGITA MAGSIYPGQA SLLDQTDSWN HRPQEIAMWQ PNVEDSTVLD 1020
1021 LRGIGQVLPT HLMEERLIRQ QQEMEEDQRW LEKEERFLKP DVRLSRGSID REDGSLQGPI 1080
1081 GNQHIYQPVG KPDPAAPPKK PPRPGAPGHL GSLASLSSPA DSYNEGVKLQ PQEISPPPTA 1140
1141 NLDRSNDKVY ENVTGLVKAV IEMSSKIQPA PPEEYVPMVK EVGLALRTLL ATVDETIPLL 1200
1201 PASTHREIEM AQKLLNSDLG ELINKMKLAQ QYVMTSLQQE YKKQMLTAAH ALAVDAKNLL 1260
1261 DVIDQARLKM LGQTRPH                                              1320
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1-218: GST **Pink:** Thrombin cleavage site **blue**: FAK **boxed**:variation from RefSeq

FAK wt[1] Amino Acid Sequence

```
   1 MAAAYLDPNL NHTPNSSTKT HLGTGMERSP GAMERVLKVF HYFESNSEPT TWASIIRHGD  60
  61 ATDVRGIIQK IVDSHKVKHV ACYGFRLSHL RSEEVHWLHV DMGVSSVREK YELAHPPEEW 120
 121 KYELRIRYLP KGFLNQFTED KPTLNFFYQQ VKSDYMLEIA DQVDQEIALK LGCLEIRRSY 180
 181 WEMRGNALEK KSNYEVLEKD VGLKRFFPKS LLDSVKAKTL RKLIQQTFRQ FANLNREESI 240
 241 LKFFEILSPV YRFDKECFKC ALGSSWIISV ELAIGPEEGI SYLTDKGCNP THLADFTQVQ 300
 301 TIQYSNSEDK DRKGMLQLKI AGAPEPLTVT APSLTIAENM ADLIDGYCRL VNGTSQSFII 360
 361 RPQKEGERAL PSIPKLANSE KQGMRTHAVS VSETDDYAEI IDEEDTYTMP STRDYEIQRE 420
 421 RIELGRCIGE GQFGDVHQGI YMSPENPALA VAIKTCKNCT SDSVREKFLQ EALTMRQFDH 480
 481 PHIVKLIGVI TENPVWIIME LCTLGELRSF LQVRKYSLDL ASLILYAYQL STALAYLESK 540
 541 RFVHRDIAAR NVLVSSNDCV KLGDFGLSRY MEDSTYYKAS KGKLPIKWMA PESINFRRFT 600
 601 SASDVWMFGV CMWEILMHGV KPFQGVKNND VIGRIENGER LPMPPNCPPT LYSLMTKCWA 660
 661 YDPSRRPRFT ELKAQLSTIL EEEKAQQEER MRMESRRQAT VSWDSGGSDE APPKPSRPGY 720
 721 PSPRSSEGFY PSPQHMVQTN HYQVSGYPGS HGITAMAGSI YPGQASLLDQ TDSWNHRPQE 780
 781 IAMWQPNVED STVLDLRGIG QVLPTHLMEE RLIRQQQEME EDQRWLEKEE RFLKPDVRLS 840
 841 RGSIDREDGS LQGPIGNQHI YQPVGKPDPA APPKKPPRPG APGHLGSLAS LSSPADSYNE 900
 901 GVKLQPQEIS PPPTANLDRS NDKVYENVTG LVKAVIEMSS KIQPAPPEEY VPMVKEVGLA 960
 961 LRTLLATVDE TIPLLPASTH REIEMAQKLL NSDLGELINK MKLAQQYVMT SLQQEYKKQM 1020
1021 LTAAHALAVD AKNLLDVIDQ ARLKMLGQTR PH                              1080
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blue: FAK sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_722560.1

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