

FAK (non activated)

focal adhesion kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: PTK2

Synonyms: FADK, FADK1, FAK1, FRNK, pp125FAK

Product No.: 0165-0000-1

Lot: 007

Description: Human FAK, amino acids A₂-H₁₀₅₂ (as in NCBI/Protein entry NP_722560.1), N-terminal GST fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: FAK Lot 007, was confirmed as FAK by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 145,394 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

Storage buffer: 50 mM Tris-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 4 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!

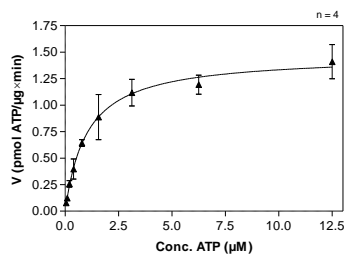
Protein concentration: 0.237 µg/µl

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

Biochemical Parameters:

Specific kinase activity (P_i transfer): 1.5 pmol/µg × min

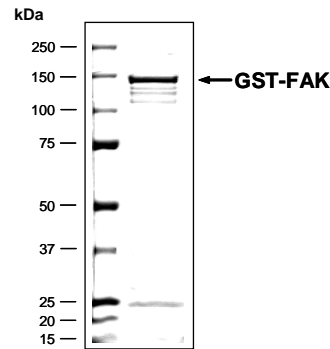
ATP-K_M: 1 µM



Determination of K_M value & Specific activity:

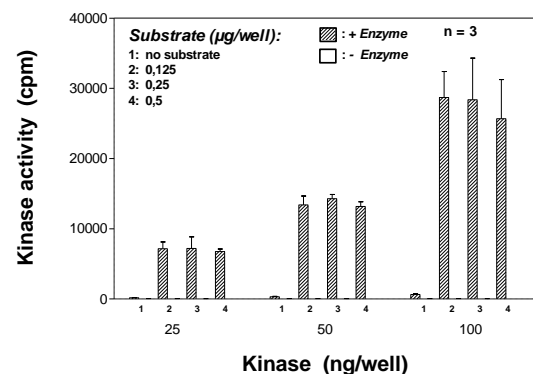
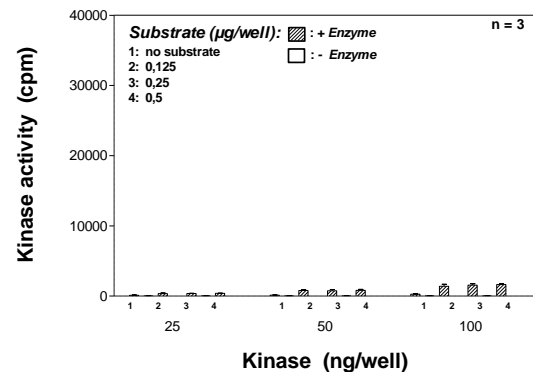
- Assay conditions:
 - 60 mM HEPES-NaOH, pH 7.5
 - 3 mM MgCl₂
 - 3 mM MnCl₂
 - 3 µM Na-orthovanadate
 - 1.2 mM DTT
 - 50 µg/ml PEG_{20,000}
 - ATP (variable)
 - Substrate: Poly(Glu/Tyr)_{4,1}, 5 µg/ml
 - Kinase: 4 µg/ml
- Filter binding assay
 - MSFC membrane (Millipore)

**FAK Lot 007:
Coomassie stain**



2.0 µg GST-FAK

Kinase activity FAK (non activated) vs. active FAK:



Final assay concentrations:

- 60 mM HEPES-NaOH, pH 7.5
 - 3 mM MgCl₂
 - 3 mM MnCl₂
 - 3 µM Na-orthovanadate
 - 1.2 mM DTT
 - 50 µg / ml PEG_{20,000}
 - 1 µM ATP
 - Substrate (variable): Poly(Glu:Tyr)_{4,1}
 - Recombinant FAK (non activated) or active FAK: 4 µg/ml
- Assay: ³³PanQinase® Assay**

FAK

Product No.: 0165-0000-1

GST-FAK Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	LVPRGSAAAY	LDPNLNHTPN	240
241	SSTKTHLGTG	MERSPGAMER	VLKVFHYFES	NSEPTTWASI	IRHGATDVR	GIIQKIVDSH	300
301	KVKHVACYGF	RLSHLRSEEV	HWLHVDMGVS	SVREKYELAH	PPEEWKYELR	IRYLPKGFLN	360
361	QFTEDKPTLN	FFYQQVKS DY	MLEIADQVDQ	EIALKLGCLE	IRRSYWEMRG	NALEKKS NYE	420
421	VLEKDVGLKR	FFPKSLLDSV	KAKTLRKL IQ	QTFRQFANLN	REESILKFFE	ILSPVYRFDK	480
481	ECFKCALGSS	WIISVELAIG	PEEGISY LTD	KGCNPTH LAD	FTQVQTIQYS	NSEDKDRKGM	540
541	LQLKIAGAPE	PLTVTAPSLT	IAENMADLID	GYCRLVNGTS	QSFII RPQKE	GERALPSIPK	600
601	LANSEKQGM R	THAVSVSETD	DYAEI IDEED	TYTMPSTRDY	EIQRERIE LG	RCIGEGQFGD	660
661	VHQGIYMSPE	NPALAVAIKT	CKNCTSDSV R	EKFLQEAL TM	RQFDHPHIVK	LIGVITENPV	720
721	WIIMELCTLG	ELRSFLQVRK	YSLDLASLIL	YAYQLSTALA	YLESKRFVHR	DIAARNVLVS	780
781	SNDCVKLGDF	GLSRYMEDST	YKASKGKLP	IKWMAPE SIN	FRRFTSASDV	WMFGVCMWEI	840
841	LMHGVPFQG	VKNNDVIGRI	ENGERLPMPP	NCPPTLYSLM	TKCWAYDPSR	RPRFTELK AQ	900
901	LSTILEE EKA	QOEERMRMES	RRQATVSWDS	GSDEAPPKP	SRPGYSPRS	SEGFYSPSQH	960
961	MVQTNHYQVS	GYPGSHGITA	MAGSIYPGQA	SLLDQTD SWN	HRPQEIAMWQ	PNVEDSTVLD	1020
1021	LRGIGQVLP T	HLMEERLIRQ	QOEMEEDQRW	LEKEERFLKP	DVRLSRGSID	REDGSLQGP I	1080
1081	GNQHIYQPVG	KPDPAAPPKK	PPRPGAPGHL	GSLASLSSPA	DSYNEGVLQ	PQEISPPPTA	1140
1141	NLDRSNDKVY	ENVTGLVKAV	IEMSSKIQPA	PPEEYVPMVK	EVGLALRTLL	ATVDETIPLL	1200
1201	PASTHREIEM	AOKLLNSDLG	ELINKMKLAQ	QYVMTSLQOE	YKKQMLTAAH	ALAVDAKNLL	1260
1261	DVIDQARLKM	LGQTRPH					1320

1-218: GST **Pink**: Thrombin cleavage site **blue**: FAK **boxed**: variation from RefSeq

FAK wt ¹ Amino Acid Sequence							
1	M AAAYLDPNL	N HTPNSSTKT	H LGTGMERSP	G AMERVLKVF	H YFESNSEPT	T WASIIRHGD	60
61	A TDVRGIIQK	I VDSHKVKHV	A CYGFRLSHL	R SEEVHVLHV	D MGVSSVREK	Y ELAHPPEEW	120
121	K YELRIRYLP	K GFLNQFTED	K PTLNFFFYQQ	V KSDYMLEIA	D QVDQEIALK	L GCLEIRRSY	180
181	W EMRGNALPK	K SNYEVLEKD	V GLKRFFPKS	L LDVSKATL	R KLIQQTFRQ	F ANLNREESI	240
241	L KFFEILSPV	Y RFDKECFKC	A LGSSWIIISV	E LAIGPEEGI	S YLTDKGCNP	T HLADFTQVQ	300
301	T IQYSNSEDK	D RKGMQLKI	A GAPEPLTVT	A PSLTIAENM	A DLIDGYCRL	V NGTSQSFI I	360
361	R PQKEGERAL	P SIPKLANSE	K QGMRTHAVS	V SETDDYAEI	I DEEDTYTMP	S TRDYEIQRE	420
421	R IELGRGIGE	G QFGDVHQGI	Y MSPENPALA	V AIKTCKNCT	S DSVREKFLQ	E ALTMRQFDH	480
481	P HIVKLIGVI	T ENPVWIIME	L CTLGELRSF	L QVRKYSLDL	A SLILYAYQL	S TALAYLESK	540
541	R FVHRDIAAR	N VLVSSNDCV	K LGDFGLSRY	M EDSTYYKAS	K GKLPKWKMA	P ESINFRRFT	600
601	S ASDVWMFGV	C MWEILMHGV	K PFQGVKNND	V IGRIENGER	L MPMPNCPPT	L YSLMTCWA	660
661	Y DPSRRPRFT	E LKAQLSTIL	E EEKAQQEER	M RMESRRQAT	V SWDSGGSDE	A PPKPSRPGY	720
721	P SPRSSEGFY	P SPQHMVQTN	H YQVSGYPGS	H GITAMAGSI	Y PGQASLLDQ	T DSWNHRPQE	780
781	I AMWQPNVED	S TVLDRGIG	Q VLPTHLMEE	R LIRQQQEME	E DQRWLEKEE	R FLKPDVRLS	840
841	R GSIDREDGS	L QGPIGNQHI	Y QVPGKPDPA	A PPKKPRPG	A PGHLGSLAS	L SSPADSYNE	900
901	G VKLQPQEIS	P PPTANLDRS	N DKVYENVTG	L VKAVIEMSS	K IQPAPPEEY	V PMVKEVGLA	960
961	L RLLATVDE	T IPLLPASTH	R EIEMAQKLL	N SDLGELINK	M KLAQQYVMT	S LQOEYKQOM	1020
1021	L TAAHALAVD	A KNLLDVIDQ	A RLKMLGQTR	P H			1080

blue: FAK sequence expressed in recombinant protein

¹NCBI/Protein accession number NP_722560.1