

Certificate of Analysis



HIPK3

homeodomain interacting protein kinase 3

Recombinant Human Active Protein Kinase

HGNC Symbol: HIPK3

Synonyms: ANPK, DYRK6, FIST, FIST3, PKY, YAK1

Product No.: 0936-0000-1

Lot: 004

Description: Human HIPK3, internal fragment, amino acids T₁₆₃-N₅₆₂ (as in NCBI/Protein entry NP_005725.3), activated, untagged, expressed in Sf9 insect cells

Product identity: HIPK3 Lot 004, was confirmed as HIPK3 by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Protein}: 46,163 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography, followed by cleavage of GST-tag

Activation: In vitro autoactivation

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

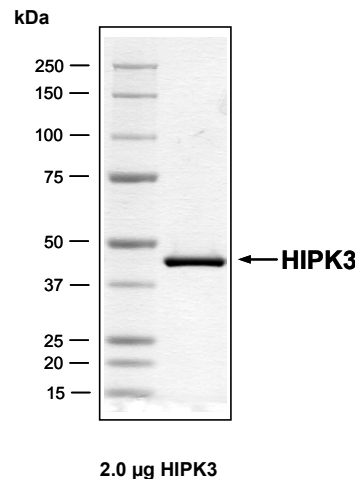
Storage temperature: -80°C
Avoid repeated freeze-thaw cycles!

Protein concentration: 0.094 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

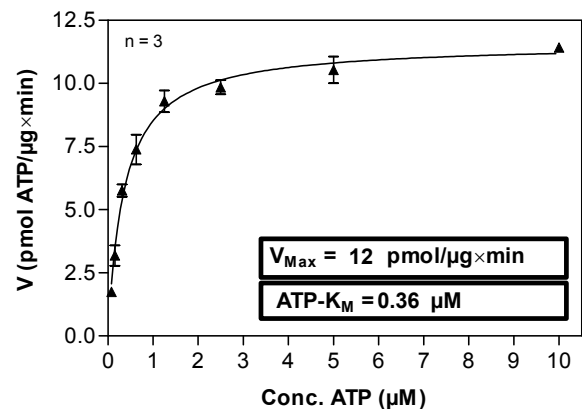
Biochemical Parameters:

Specific activity: 12 pmol/µg×min
ATP-K_M: 0.4 µM

**HIPK3 Lot 004:
Coomassie stain**



**HIPK3 Lot 004:
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 MgCl₂
 - 3 mM MnCl₂
 - 3 µM Na-orthovanadate
 - 1.2 mM DTT
 - 50 µg / ml PEG_{20,000}
 - ATP (variable)
 - Substrate: RBER-IRStide, 100 µg / ml
 - HIPK3: 1.0 µg / ml
- Filter binding assay
 - MSFC membrane (Millipore)

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HIPK3

Product No.: 0936-0000-1

HIPK3 Recombinant Fusion Protein Amino Acid Sequence										
1	GPLAMG	TVVT	ATTGSKQ	NCT	TGEDYQL	VQ	HEVLCSMKNT	YEVLDFLGRG	TFGQVVKCWK	60
61	RGTNEIVA	IK	ILKNHPS	YAR	QGQIEV	SILA	RLSTENADEY	NFVRAYECFQ	HRNHTCLVFE	120
121	MLEQNLYD	FL	KQNKFS	SPLPL	KVIRPIL	QQV	ATALKKLSL	GLIHADLKPE	NIMLVDPVRQ	180
181	PYRVKVID	FG	SASHVSK	TVC	STYLQSR	YYR	APEIILGLPF	CEAIDMWSLG	CVIAELFLGW	240
241	PLYPGALE	YD	QIRYISQ	TQG	LPGEQL	LVNG	TKSTRFFCKE	TDMSHSGWRL	KTLEEHEAET	300
301	GMKSKEAR	KY	IFNSLDD	VAH	VNTVMD	LEGS	DLAELKADRR	EFVSLKML	LIDADLRITP	360
361	AETLNHPF	VN	MKHLDF	PHS	NHVKSC	FHIM	DICKSHLNSC	DTNNH		420

1-6: legacy from 3C cleavage blue: HIPK3 fragment

HIPK3 wt ¹ amino acid sequence										
1	MASQVLV	YPP	YVYQTQ	SSAF	CSVKKL	KVEP	SSCVFQERNY	PRTYVNGRNF	GNSHPPTKGS	60
61	AFQTKIP	FN	PRGHNF	SLOT	SAVVLK	NTAG	ATKVIAAAQ	QAHVQAPQIG	AWRNLHFLE	120
121	GPQRCGL	KRK	SEELDN	HSSA	MQIVDEL	SIL	PAMLQTNMGN	PVTVVTATTG	SKQNTTGEG	180
181	DYLVQHE	VL	CSMKNT	YEV	DFLGRG	TFGQ	VVKCWRGTN	EIVAIIKLN	HPSYARQGI	240
241	EVSILAR	LST	ENADEYN	FVR	AYECFQ	HRNH	TCLVFEMLEQ	NLYDFLKQNK	FSPLPLKVI	300
301	PILQQVA	TAL	KKLSLGL	LIH	ADLKPEN	IML	VDPVRQPYRV	KVIDFGSASH	VSKTVCSTYL	360
361	QSRYR	RAPEI	ILGLPF	CEAI	DMWSLG	CVIA	ELFLGWPLYP	GALEYDQIRY	ISQTQGLPGE	420
421	QLLVNGT	KST	RFFCKET	DMS	HSGWRL	KTLE	EHEAETGMKS	KEARKYIFNS	LDDVAHVNTV	480
481	MDLEGS	DLA	EKADRRE	FVS	LLKKML	LIDA	DLRITPAETL	NHPFVNMKHL	LDFPHSNHVK	540
541	SCFHIM	DICK	SHLNSC	DTNN	HNKTSLL	RPV	ASSSTATLTA	NFTKIGTLRS	QALTTSAHSV	600
600	VHGGIPL	QAG	TAQFGC	GDAF	QQTLLI	ICPPA	IQGIPATHGK	PTSYSIRVDN	TVPLVTQAPA	660
661	VQPLQIR	PGV	LSQTWS	GRTQ	QMLVPA	WQQV	TPLAPATTTL	TSESVAGSHR	LGDWGKMISC	720
721	SNHYNS	VMPQ	PLLTNQ	ITLS	APQPV	SVGIA	HVVWPQPATT	KKNKQCQNRG	ILVKLMEWEP	780
781	GREEINA	FSW	SNSLQNT	NIP	HSAFISP	KII	NGKDVEEVSC	IETQDNQNSE	GEARNCCETS	840
841	IRQDSD	SSVS	DKQRQT	IIIA	DSPSPAV	SVI	TISSDTDEEE	TSQRHSLREC	KGSLDCEACQ	900
901	STLNIDR	MCS	LSSPDST	LST	SSSQSS	PSP	CKRPNSMSDE	EQESSCDTV	GSPTSDSSGH	960
961	DSPFAES	TFV	EDTHE	TELV	SSADTET	KPA	VCSVVVPPVE	LENGLNADEH	MANTDSICQP	1020
1021	LIKGRS	APGR	LNQPSA	VGTR	QQKLTS	AFQQ	QHLNFSQVQH	FGSGHQEWNG	NFGHRRQQAY	1080
1081	IPTSVT	SNPF	TLSHG	SPNHT	AVHAHL	AGNT	HLGGQPTLLP	YPSSATLSSA	APVAHLLASP	1140
1141	CTSRPML	QHP	TYNISH	PSGI	VHQVPV	GLNP	RLLPSPTIHQ	TQYKPIFPPH	SYIAASPAYT	1200
1201	GFPLSPT	KLK	QYPYM							1260

blue: HIPK3 sequence expressed in fusionprotein

¹NCBI/Protein accession number NP_005725.3