Certificate of Analysis

REACTION BIOLOGY

ProQinase™ 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 T_{163}-N_{562} (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

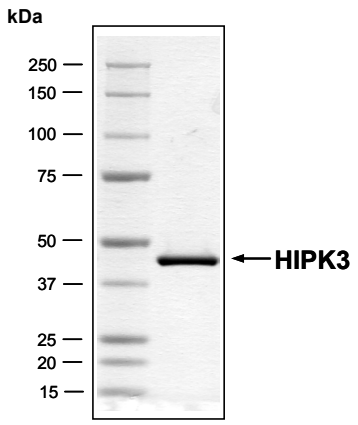
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 20% glycerol

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)

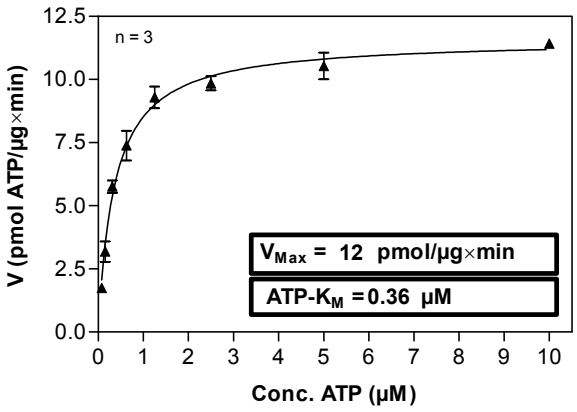
Biochemical Parameters:
Specific activity: 12 pmol/µg×min
ATP-K_M: 0.4 µM

HIPK3 Lot 004:
Coomassie stain



2.0 µg HIPK3

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 $MgCl_2$
  3 mM $MnCl_2$
  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)

Recombinant Proteins

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ProQinase™ HIPK3

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HIPK3 Recombinant Fusion Protein Amino Acid Sequence

1	GPLAMG**TVVT**	**ATTGSKQNCT**	**TGEGDYQLVQ**	**HEVLCSMKNT**	**YEVLDFLGRG**	**TFGQVVKCWK**	60
61	**RGTNEIVAIK**	**ILKNHPSYAR**	**QGQIEVSILA**	**RLSTENADEY**	**NFVRAYECFQ**	**HRNHTCLVFE**	120
121	**MLEQNLYDFL**	**KQNKFSPLPL**	**KVIRPILQQV**	**ATALKKLKSL**	**GLIHADLKPE**	**NIMLVDPVRQ**	180
181	**PYRVKVIDFG**	**SASHVSKTVC**	**STYLQSRYYR**	**APEIILGLPF**	**CEAIDMWSLG**	**CVIAELFLGW**	240
241	**PLYPGALEYD**	**QIRYISQTQG**	**LPGEQLLNVG**	**TKSTRFFCKE**	**TDMSHSGWRL**	**KTLEEHEAET**	300
301	**GMKSKEARKY**	**IFNSLDDVAH**	**VNTVMDLEGS**	**DLLAEKADRR**	**EFVSLLKKML**	**LIDADLRITP**	360
361	**AETLNHPFVN**	**MKHLLDFPHS**	**NHVKSCFHIM**	**DICKSHLNSC**	**DTNNHN**		420

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

HIPK3 wt[1] amino acid sequence

1	MASQVLVYPP	YVYQTQSSAF	CSVKKLKVEP	SSCVFQERNY	PRTYVNGRNF	GNSHPPTKGS	60
61	AFQTKIPFNR	PRGHNFSLQT	SAVVLKNTAG	ATKVIAAQAQ	QAHVQAPQIG	AWRNRLHFLE	120
121	GPQRCGLKRK	SEELDNHSSA	MQIVDELSIL	PAMLQTNMGN	PV**TVVTATTG**	**SKQNCTTGEG**	180
181	**DYQLVQHEVL**	**CSMKNTYEVL**	**DFLGRGTFGQ**	**VVKCWKRGTN**	**EIVAIKILKN**	**HPSYARQGQI**	240
241	**EVSILARLST**	**ENADEYNFVR**	**AYECFQHRNH**	**TCLVFEMLEQ**	**NLYDFLKQNK**	**FSPLPLKVIR**	300
301	**PILQQVATAL**	**KKLKSLGLIH**	**ADLKPENIML**	**VDPVRQPYRV**	**KVIDFGSASH**	**VSKTVCSTYL**	360
361	**QSRYYRAPEI**	**ILGLPFCEAI**	**DMWSLGCVIA**	**ELFLGWPLYP**	**GALEYDQIRY**	**ISQTQGLPGE**	420
421	**QLLNVGTKST**	**RFFCKETDMS**	**HSGWRLKTLE**	**EHEAETGMKS**	**KEARKYIFNS**	**LDDVAHVNTV**	480
481	**MDLEGSDLLA**	**EKADRREFVS**	**LLKKMLLIDA**	**DLRITPAETL**	**NHPFVNMKHL**	**LDFPHSNHVK**	540
541	**SCFHIMDICK**	**SHLNSCDTNN**	**HN**KTSLLRPV	ASSSTATLTA	NFTKIGTLRS	QALTTSAHSV	600
600	VHHGIPLQAG	TAQFGCGDAF	QQTLIICPPA	IQGIPATHGK	PTSYSIRVDN	TVPLVTQAPA	660
661	VQPLQIRPGV	LSQTWSGRTQ	QMLVPAWQQV	TPLAPATTTL	TSESVAGSHR	LGDWGKMISC	720
721	SNHYNSVMPQ	PLLTNQITLS	APQPVSVGIA	HVVWPQPATT	KKNKQCQNRG	ILVKLMEWEP	780
781	GREEINAFSW	SNSLQNTNIP	HSAFISPKII	NGKDVEEVSC	IETQDNQNSE	GEARNCCETS	840
841	IRQDSDSSVS	DKQRQTIIIA	DSPSPAVSVI	TISSDTDEEE	TSQRHSLREC	KGSLDCEACQ	900
901	STLNIDRMCS	LSSPDSTLST	SSSGQSSPSP	CKRPNSMSDE	EQESSCDTVD	GSPTSDSSGH	960
961	DSPFAESTFV	EDTHENTELV	SSADTETKPA	VCSVVVPPVE	LENGLNADEH	MANTDSICQP	1020
1021	LIKGRSAPGR	LNQPSAVGTR	QQKLTSAFQQ	QHLNFSQVQH	FGSGHQEWNG	NFGHRRQQAY	1080
1081	IPTSVTSNPF	TLSHGSPNHT	AVHAHLAGNT	HLGGQPTLLP	YPSSATLSSA	APVAHLLASP	1140
1141	CTSRPMLQHP	TYNISHPSGI	VHQVPVGLNP	RLLPSPTIHQ	TQYKPIFPPH	SYIAASPAYT	1200
1201	GFPLSPTKLS	QYPYM					1260

blue: HIPK3 sequence expressed in fusionprotein

[1]NCBI/Protein accession number NP_005725.3

Recombinant Proteins