

# Certificate of Analysis

## PAK6

p21 protein (Cdc42/Rac)-activated kinase 6

Recombinant Human Active Protein Kinase

HGNC Symbol: PAK6

Synonyms: PAK5

Product No.: 0423-0000-1

Lot: 001

**Description:** Human PAK6, full length, amino acids M<sub>1</sub>-C<sub>681</sub> (as in NCBI/Protein entry NP\_064553.1), N-terminal GST-HIS<sub>6</sub> fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

**Product identity:** PAK6 Lot 001, was confirmed as PAK6 by mass spectroscopy LC-ESI-MS/MS

**Theoretical MW**<sub>Fusion Protein</sub>: 104,780 Da

**Expression:** Baculovirus infected Sf9 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, 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:** 0.082 µg/µl

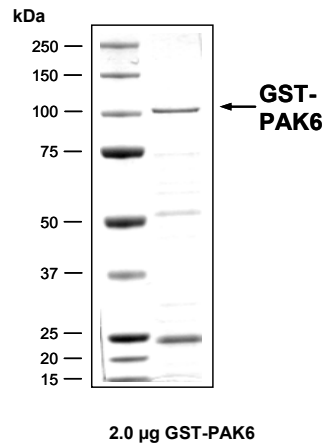
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

### Biochemical Parameters:

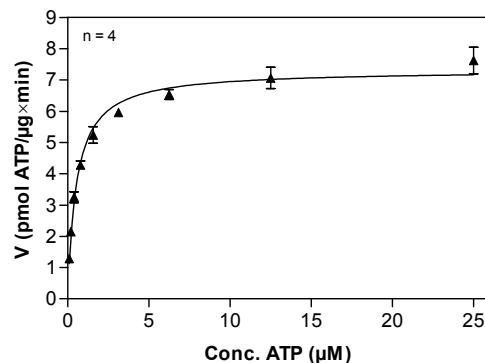
Specific kinase activity (P<sub>i</sub> transfer): 7.3 pmol/µg×min

ATP-K<sub>M</sub>: 0.5 µM

### PAK6 Lot 001: Coomassie stain



### PAK6 Lot 001: Determination of V<sub>max</sub> and K<sub>M</sub> value for ATP



### Determination of K<sub>M</sub> value & Specific activity:

- Assay conditions:
  - 60 mM HEPES-NaOH, pH 7.5
  - 3 mM MgCl<sub>2</sub>
  - 3 mM MnCl<sub>2</sub>
  - 3 µM Na-orthovanadate
  - 1.2 mM DTT
  - 50 µg / ml PEG<sub>20,000</sub>
  - ATP (variable)
  - Substrate: tetra(LRRWSLG), 30 µg/ml
  - Kinase: 4.0 µg / ml
- Filter binding assay
  - MSPH membrane (Millipore)

### Additional assay technology: PAK6 Lot 001

was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from



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PAK6 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG <b>HHHHHH</b> HG	RRRASVAAGI	240
241	<b>LVPRGS</b> PGLD	GIYARGIQAS	<b>MFRKKKKR</b> P	<b>EISAPQNFQ</b> H	<b>RVHTSFDP</b> KE	<b>GKFVGLPP</b> QW	300
301	<b>QNILDTLRR</b> P	<b>KPVVDPSR</b> IT	<b>RVQLQPMK</b> TV	<b>VRGSAMPV</b> DG	<b>YISGLLND</b> IQ	<b>KLSVISSN</b> TL	360
361	<b>RGRSPTSRR</b> R	<b>AQSLGLLG</b> DE	<b>HWATDPDM</b> YL	<b>QSPQSER</b> TD	<b>HGLYLSCN</b> GG	<b>TPAGHKQMP</b> W	420
421	<b>PEPQSPRV</b> LP	<b>NGLAAQAQ</b> SL	<b>GPAEFQGA</b> SQ	<b>RCLQLGAC</b> LQ	<b>SSPPGASP</b> PT	<b>GTNRHGMKA</b> A	480
481	<b>KHGSEEAR</b> PQ	<b>SCLVGSAT</b> GR	<b>PGGEGSPS</b> PK	<b>TRESSLKR</b> RL	<b>FRSMFLST</b> A	<b>TAPSSSSK</b> PG	540
541	<b>PPPQSKPN</b> SS	<b>FRPPQKDN</b> PP	<b>SLVAKAQSL</b> P	<b>SDQPVGTF</b> S	<b>LTTSDTSS</b> PQ	<b>KSLRTA</b> <b>I</b> ATG	600
601	<b>QLPGRSSP</b> AG	<b>SPRTWHAQ</b> IS	<b>TSNLYLPQ</b> DP	<b>TVAKGAL</b> AGE	<b>DTGVVTHE</b> QF	<b>KAALRMVV</b> DQ	660
661	<b>GDPRLLLD</b> SY	<b>VKIGEGST</b> GI	<b>VCLAREKH</b> SG	<b>RQVAVKMM</b> DL	<b>RKQQRRELL</b> F	<b>NEVVIMRD</b> YQ	720
721	<b>HFNVVEMY</b> KS	<b>YLVEELWV</b> L	<b>MEFLQGG</b> ALT	<b>DIVSQVRL</b> NE	<b>EQIATVCE</b> AV	<b>LQALAYLH</b> AQ	780
781	<b>GVIHRDIK</b> SD	<b>SILLTLDG</b> RV	<b>KLSDFGFC</b> AQ	<b>ISKDVPKR</b> KS	<b>LVGTPYWM</b> AP	<b>EVISRSLY</b> AT	840
841	<b>EVDIWSLG</b> IM	<b>VIEMVDGE</b> PP	<b>YFSDSPVQ</b> AM	<b>KRLRDSPP</b> PK	<b>LKNSHKVSP</b> V	<b>LRDFLERML</b> V	900
901	<b>RDPQERAT</b> AQ	<b>ELLDHPFL</b> LQ	<b>TGLPECLV</b> PL	<b>IQLYRKQT</b> ST	<b>C</b>		960

1-218: GST    **Red:** HIS6-tag    **Pink:** Thrombin cleavage site    **blue:**PAK6    **boxed:**variation from RefSeq

PAK6 wt <sup>1</sup> Amino Acid Sequence							
1	<b>MFRKKKKR</b> P	<b>EISAPQNF</b> Q	<b>RVHTSFDP</b> KE	<b>GKFVGLPP</b> QW	<b>QNILDTLRR</b> P	<b>KPVVDPSR</b> IT	60
61	<b>RVQLQPMK</b> TV	<b>VRGSAMPV</b> DG	<b>YISGLLND</b> IQ	<b>KLSVISSN</b> TL	<b>RGRSPTSRR</b> R	<b>AQSLGLLG</b> DE	120
121	<b>HWATDPDM</b> YL	<b>QSPQSER</b> TD	<b>HGLYLSCN</b> GG	<b>TPAGHKQMP</b> W	<b>PEPQSPRV</b> LP	<b>NGLAAQAQ</b> SL	180
181	<b>GPAEFQGA</b> SQ	<b>RCLQLGAC</b> LQ	<b>SSPPGASP</b> PT	<b>GTNRHGMKA</b> A	<b>KHGSEEAR</b> PQ	<b>SCLVGSAT</b> GR	240
241	<b>PGGEGSPS</b> PK	<b>TRESSLKR</b> RL	<b>FRSMFLST</b> A	<b>TAPSSSSK</b> PG	<b>PPPQSKPN</b> SS	<b>FRPPQKDN</b> PP	300
301	<b>SLVAKAQSL</b> P	<b>SDQPVGTF</b> S	<b>LTTSDTSS</b> PQ	<b>KSLRTA</b> <b>P</b> ATG	<b>QLPGRSSP</b> AG	<b>SPRTWHAQ</b> IS	360
361	<b>TSNLYLPQ</b> DP	<b>TVAKGAL</b> AGE	<b>DTGVVTHE</b> QF	<b>KAALRMVV</b> DQ	<b>GDPRLLLD</b> SY	<b>VKIGEGST</b> GI	420
421	<b>VCLAREKH</b> SG	<b>RQVAVKMM</b> DL	<b>RKQQRRELL</b> F	<b>NEVVIMRD</b> YQ	<b>HFNVVEMY</b> KS	<b>YLVEELWV</b> L	480
481	<b>MEFLQGG</b> ALT	<b>DIVSQVRL</b> NE	<b>EQIATVCE</b> AV	<b>LQALAYLH</b> AQ	<b>GVIHRDIK</b> SD	<b>SILLTLDG</b> RV	540
541	<b>KLSDFGFC</b> AQ	<b>ISKDVPKR</b> KS	<b>LVGTPYWM</b> AP	<b>EVISRSLY</b> AT	<b>EVDIWSLG</b> IM	<b>VIEMVDGE</b> PP	600
601	<b>YFSDSPVQ</b> AM	<b>KRLRDSPP</b> PK	<b>LKNSHKVSP</b> V	<b>LRDFLERML</b> V	<b>RDPQERAT</b> AQ	<b>ELLDHPFL</b> LQ	660
661	<b>TGLPECLV</b> PL	<b>IQLYRKQT</b> ST	<b>C</b>				720

**blue:** PAK6 sequence expressed in fusion protein    **Red:** variant in fusion protein

<sup>1</sup>NCBI/Protein accession number NP\_064553.1  
P<sub>337</sub>L: SNP variation see NCBI/dbSNP ID: rs3743137