

# Certificate of Analysis



## PAK3

p21 (CDKN1A)-activated protein kinase 3

Recombinant Human Active Protein Kinase

HGNC Symbol: PAK3

**Synonyms:** CDKN1A; MRX30; OPHN3; PAK3beta; bPAK; hPAK3

**Product No.:** 0422-0000-1

**Lot:** 001

**Description:** Human PAK3, full length, amino acids M<sub>1</sub>-R<sub>544</sub> (as in NCBI/Protein entry NP\_002569.1), N-terminal GST-HIS<sub>6</sub> fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

**Product identity:** PAK3 Lot 001, was confirmed as PAK3 by PAK3 specific Western Blotting

**Theoretical MW<sub>Fusion Protein</sub>:** 90,588 Da

**Expression:** Baculovirus infected Sf9 cells

**Purification:** GST-Affinity Chromatography

**Activation:** This kinase was not activated by special procedures

**Storage buffer:** 50 mM HEPES-NaOH, pH 7.5, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20% glycerol

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

**Protein concentration:** 0.360 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): 79 pmol/µg×min  
ATP-K<sub>M</sub>: 15 µM

### Additional assay technology: PAK3 Lot 001

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

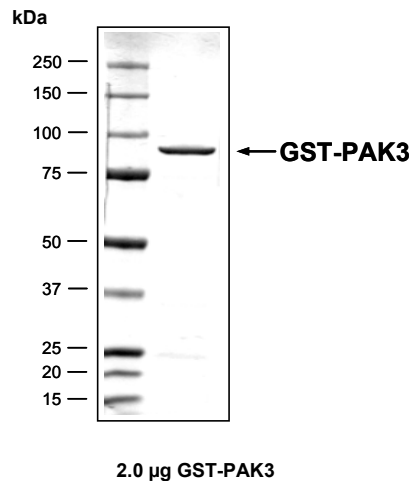


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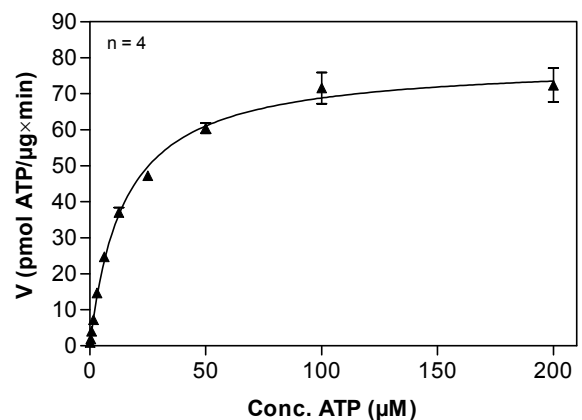
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### PAK3 Lot 001: Coomassie stain



### PAK3 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), 40 µg / ml
  - PAK3: 4.0 µg / ml
- Filter binding assay  
MSFC membrane (Millipore)

# PAK3

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PAK3 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSM	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIQID	KYLKSSKYIA	WPLQGQWATF	GGGDHPPKSD	PMGHHHHHGG	RRRASVAAGI	240
241	LVPRGSPGLD	GIYARGIQAS	MSDGLDNEEK	PPAPPLRMNS	NNRDSSALNH	SSKPLPMAPE	300
301	EKNKKARLRS	IFPGGGDKTN	KKKEKERPEI	SLPSDFEHTI	HVGFDVAVTGE	FTGIPEQWAR	360
361	LLQTSNITKL	EQKKNPQAVL	DVLKFYDSKE	TVNNQKYSF	TSGDKSAHGY	IAAHPSSTKT	420
421	ASEPPLAPPV	SEEEEEEEEE	EEDENEPPP	IAPRPEHTKS	IYTRSVVESI	ASPAVPNKEV	480
481	TPPSAENANS	STLYRNTDRQ	RKKSMTDEE	ILEKLSIVS	VGDPKKKYTR	FEKIQGASG	540
541	TVYTALDIAT	GQEVAIQMN	LQQQPKKELI	INEILVMREN	KNPNIVNYLD	SYLVGDELWV	600
601	VMEYLAGGSL	TDVVTETCMD	EGQIAAVCRE	CLQALDFLHS	NQVIHRDIKS	DNILLGMDGS	660
661	VKLTDFGFCA	QITPEQSKRS	TMVGTPYWMA	PEVVTRKAYG	PKVDIWSLGI	MAIEMVEGEP	720
721	PYLNENPLRA	LYLIATNGTP	ELQNERLSA	VFRDFLNRCL	EMDVDRRGS	KELLQHPFLK	780
781	LAKPLSSLTP	LIIAAKEAIK	NSSR				840

1-218: GST Red: HIS6-tag Pink: Thrombin cleavage site blue: PAK3

PAK3 wt <sup>1</sup> amino acid sequence							
1	MSDGLDNEEK	PPAPPLRMNS	NNRDSSALNH	SSKPLPMAPE	EKNKKARLRS	IFPGGGDKTN	60
61	KKKEKERPEI	SLPSDFEHTI	HVGFDVAVTGE	FTGIPEQWAR	LLQTSNITKL	EQKKNPQAVL	120
121	DVLKFYDSKE	TVNNQKYSF	TSGDKSAHGY	IAAHPSSTKT	ASEPPLAPPV	SEEEEEEEEE	180
181	EEDENEPPP	IAPRPEHTKS	IYTRSVVESI	ASPAVPNKEV	TPPSAENANS	STLYRNTDRQ	240
241	RKKSMTDEE	ILEKLSIVS	VGDPKKKYTR	FEKIQGASG	TVYTALDIAT	GQEVAIQMN	300
301	LQQQPKKELI	INEILVMREN	KNPNIVNYLD	SYLVGDELWV	VMEYLAGGSL	TDVVTETCMD	360
361	EGQIAAVCRE	CLQALDFLHS	NQVIHRDIKS	DNILLGMDGS	VKLTDFGFCA	QITPEQSKRS	420
421	TMVGTPYWMA	PEVVTRKAYG	PKVDIWSLGI	MAIEMVEGEP	PYLNENPLRA	LYLIATNGTP	480
481	ELQNERLSA	VFRDFLNRCL	EMDVDRRGS	KELLQHPFLK	LAKPLSSLTP	LIIAAKEAIK	540
541	NSSR						600

blue: PAK3 sequence expressed in fusionprotein

<sup>1</sup>NCBI/Protein accession number NP\_002569.1

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