Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ Aurora-A

aurora kinase A

Recombinant Human Active Protein Kinase

HGNC Symbol: AURKA

Synonyms: AIK, ARK1, ARK-1, AurA, AURA, BTAK, hARK1, STK15, STK6, STK7

Product No.: 0166-0000-1

Lot: 004

Description: Human Aurora A, full length, amino acids M_1-S_{403} (as in NCBI/Protein entry NP_003591.2), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: Aurora-A, Lot 004, was confirmed as Aurora-A by specific Western Blotting using anti Aurora-A antibody

Theoretical $MW_{Fusion\ Protein}$: 76,334 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

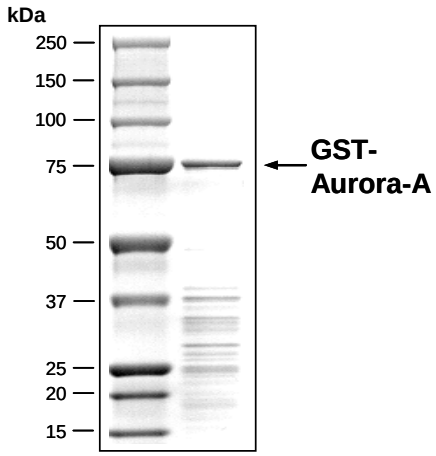
Storage buffer: 50 mM TRIS-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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

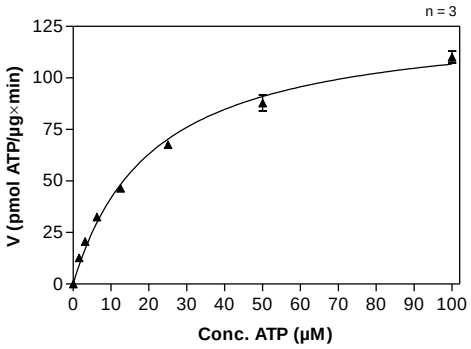
Biochemical Parameters:
Specific kinase activity (P_i transfer): 129 pmol/µg × min
ATP-K_M: 21 µM

Aurora-A Lot 004:
Coomassie stain



2.0 µg Aurora-A

Aurora-A 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: tetra(LRRLSLG) 100 µg/ml
  Kinase: 4 µg/ml
- Filter binding assay

MSPH membrane (Millipore)

Additional assay technology:
Aurora-A Lot 004 was also successfully tested by Reaction Biology for the use with the ADP-Glo™ Kinase assay from Promega ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

ProQinase™ Aurora-A

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GST-Aurora-A Recombinant Fusion Protein Amino Acid Sequence

```
   1  MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID  60
  61  GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV 120
 121  DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
 181  KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RRRASVAAGI 240
 241  LVPRGSPGLD GIYARGIQAS MGARGRMDRS KENCISGPVK ATAPVGGPKR VLVTQQFPCQ 300
 301  NPLPVNSGQA QRVLCPSNSP QRVPLQAQKL VSSHKPVQNQ KQKQLQATSV PHPVSRPLNN 360
 361  TQKSKQPLPS APENNPEEEL ASKQKNEESK KRQWALEDFE IGHPLGKGKF GNVYLAREKQ 420
 421  SKFILALKVL FKAQLEKAGV EHQLRREVEI QSHLRHPNIL RLYGYFHDAT RVYLILEYAP 480
 481  LGTVYRELQK LSKFDEQRTA TYITELANAL SYCHSKRVIH RDIKPENLLL GSAGELKIAD 540
 541  FGWSVHAPSS RRTTLCGTLD YLPPEMIEGR MHDEKVDLWS LGVLCYEFLV GKPPFEANTY 600
 601  QETYKRISRV EFTFPDFVTE GARDLISRLL KHNPSQRPML REVLEHPWIT ANSSKPSNCQ 660
 661  NKESASKQS                                                         720
```

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

Aurora-A wt[1] Amino Acid Sequence

```
   1  MDRSKENCIS GPVKATAPVG GPKRVLVTQQ FPCQNPLPVN SGQAQRVLCP SNSSQRIPLQ  60
  61  AQKLVSSHKP VQNQKQKQLQ ATSVPHPVSR PLNNTQKSKQ PLPSAPENNP EEELASKQKN 120
 121  EESKKRQWAL EDFEIGRPLG KGKFGNVYLA REKQSKFILA LKVLFKAQLE KAGVEHQLRR 180
 181  EVEIQSHLRH PNILRLYGYF HDATRVYLIL EYAPLGTVYR ELQKLSKFDE QRTATYITEL 240
 241  ANALSYCHSK RVIHRDIKPE NLLLGSAGEL KIADFGWSVH APSSRRTTLC GTLDYLPPEM 300
 301  IEGRMHDEKV DLWSLGVLCY EFLVGKPPFE ANTYQETYKR ISRVEFTFPD FVTEGARDLI 360
 361  SRLLKHNPSQ RPMLREVLEH PWITANSSKP SNCQNKESAS KQS                   420
```

blue: kinase sequence expressed in recombinant protein **Red**: variant in recombinant protein

[1]NCBI/Protein accession number NP_003591.2
I57V: SNP variation see NCBI/dbSNP ID: rs1047972
R137H: SNP variation see NCBI/dbSNP ID: rs752948413

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