Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ SRC (GST-HIS-tag)

SRC proto-oncogene, non-receptor tyrosine kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: SRC

Synonyms: ASV, SRC1

Product No.: 0200-0000-1

Lot: 005

Description: Human SRC, full length, amino acids M_1-L_{536} (as in NCBI/Protein entry NP_005408.1), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: SRC Lot 005, was confirmed as SRC by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 89,744 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

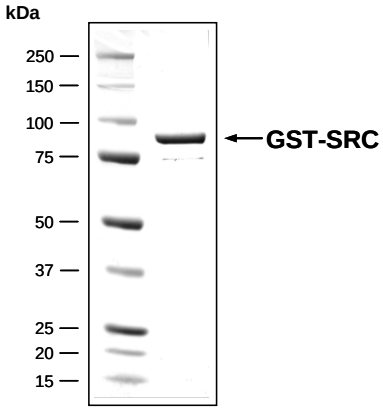
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20 % glycerol

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

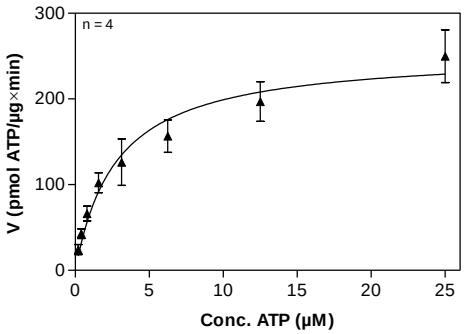
Biochemical Parameters:
Specific kinase activity (P_i transfer): 254 pmol/µg × min
ATP-K_M: 2.8 µM

SRC Lot 005:
Coomassie stain



2.0 µg GST-SRC

SRC Lot 005:
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: Poly(Glu:Tyr)$_{4:1}$, 20 µg/ml
  Kinase: 0.4 µg/ml
- Filter binding assay

MSFC membrane (Millipore)

Additional assay technology:
SRC Lot 005 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

REACTION BIOLOGY

ProQinase™ SRC (GST-HIS-tag)

Product No.: 0200-0000-1

GST-SRC 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 MASNKSKPKD ASQRRRSLEP AENVHGAGGG AFPASQTPSK 300
301 PASADGHRGP SAAFAPAAAE PKLFGGFNSS DTVTSPQRAG PLAGGVTTFV ALYDYESRTE 360
361 TDLSFKKGER LQIVNNTEGD WWLAHSLSTG QTGYIPSNYV APSDSIQAEE WYFGKITRRE 420
421 SERLLLNAEN PRGTFLVRES ETTKGAYCLS VSDFDNAKGL NVKHYKIRKL DSGGFYITSR 480
481 TQFNSLQQLV AYYSKHADGL CHRLTTVCPT SKPQTQGLAK DAWEIPRESL RLEVKLGQGC 540
541 FGEVWMGTWN GTTRVAIKTL KPGTMSPEAF LQEAQVMKKL RHEKLVQLYA VVSEEPIYIV 600
601 TEYMSKGSLL DFLKGETGKY LRLPQLVDMA AQIASGMAYV ERMNYVHRDL RAANILVGEN 660
661 LVCKVADFGL ARLIEDNEYT ARQGAKFPIK WTAPEAALYG RFTIKSDVWS FGILLTELTT 720
721 KGRVPYPGMV NREVLDQVER GYRMPCPPEC PESLHDLMCQ CWRKEPEERP TFEYLQAFLE 780
781 DYFTSTEPQY QPGENL                                               840
```

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

SRC wt[1] Amino Acid Sequence

```
  1 MGSNKSKPKD ASQRRRSLEP AENVHGAGGG AFPASQTPSK PASADGHRGP SAAFAPAAAE  60
 61 PKLFGGFNSS DTVTSPQRAG PLAGGVTTFV ALYDYESRTE TDLSFKKGER LQIVNNTEGD 120
121 WWLAHSLSTG QTGYIPSNYV APSDSIQAEE WYFGKITRRE SERLLLNAEN PRGTFLVRES 180
181 ETTKGAYCLS VSDFDNAKGL NVKHYKIRKL DSGGFYITSR TQFNSLQQLV AYYSKHADGL 240
241 CHRLTTVCPT SKPQTQGLAK DAWEIPRESL RLEVKLGQGC FGEVWMGTWN GTTRVAIKTL 300
301 KPGTMSPEAF LQEAQVMKKL RHEKLVQLYA VVSEEPIYIV TEYMSKGSLL DFLKGETGKY 360
361 LRLPQLVDMA AQIASGMAYV ERMNYVHRDL RAANILVGEN LVCKVADFGL ARLIEDNEYT 420
421 ARQGAKFPIK WTAPEAALYG RFTIKSDVWS FGILLTELTT KGRVPYPGMV NREVLDQVER 480
481 GYRMPCPPEC PESLHDLMCQ CWRKEPEERP TFEYLQAFLE DYFTSTEPQY QPGENL     540
```

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

[1]NCBI/Protein accession number NP_005408.1
G2A: legacy from cloning procedure

Recombinant Proteins