Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ MST1

serine/threonine kinase 4 (STK4)

Recombinant Human Active Protein Kinase

HGNC Symbol: STK4

Synonyms: KRS2, YSK3

Product No.: 0667-0000-1

Lot: 002

Description: Human MST1, amino acids T_3-F_{487} (as in NCBI/Protein entry NP_006273.1), N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: MST1, Lot 002, was confirmed as MST1 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 86,340 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

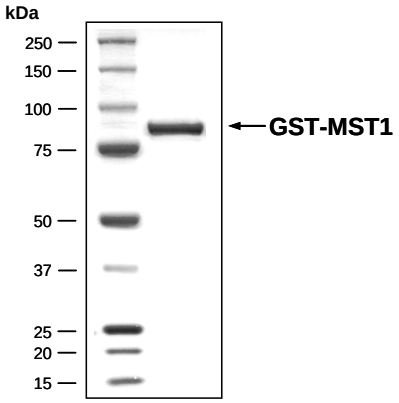
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20 % glycerol

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

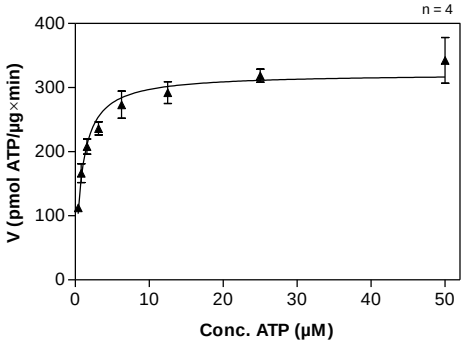
Biochemical Parameters:
Specific kinase activity (P_i transfer): 322 pmol/µg × min
ATP-K_M: 0.8 µM

MST1 Lot 002:
Coomassie stain



2.0 µg GST-MST1

MST1 Lot 002:
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: Myelin Basic Protein 100 ng/ml
  MST1: 0.5 µg/ml
- Filter binding assay

MSFC membrane (Millipore)

Additional assay technology:
MST1 Lot 002 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

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ProQinase™ MST1

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GST-MST1 Recombinant Fusion Protein Amino Acid Sequence

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  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 GIYARDSLEV LFQGPLAMLT VQLRNPPRRQ LKKLDEDSLT KQPEEVFDVL 300
301 EKLGEGSYGS VYKAIHKETG QIVAIKQVPV ESDLQEIIKE ISIMQQCDSP HVVKYYGSYF 360
361 KNTDLWIVME YCGAGSVSDI IRLRNKTLTE DEIATILQST LKGLEYLHFM RKIHRDIKAG 420
421 NILLNTEGHA KLADFGVAGQ LTDTMAKRNT VIGTPFWMAP EVIQEIGYNC VADIWSLGIT 480
481 AIEMAEGKPP YADIHPMRAI FMIPTNPPPT FRKPELWSDN FTDFVKQCLV KSPEQRATAT 540
541 QLLQHPFVRS AKGVSILRDL INEAMDVKLK RQESQQREVD QDDEENSEED EMDSGTMVRA 600
600 VGDEMGTVRV ASTMTDGANT MIEHDDTLPS QLGTMVINAE DEEEEGTMKR RDETMQPAKP 660
661 SFLEYFEQKE KENQINSFGK SVPGPLKNSS DWKIPQDGDY EFLKSWTVED LQKRLLALDP 720
721 MMEQEIEEIR QKYQSKRQPI LDAIEAKKRR QQNF                             780
```

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **Green**: 3C cleavage site **blue**: MST1 **boxed**: variation from RefSeq

MST1 wt[1] Amino Acid Sequence

```
  1 METVQLRNPP RRQLKKLDED SLTKQPEEVF DVLEKLGEGS YGSVYKAIHK ETGQIVAIKQ  60
 61 VPVESDLQEI IKEISIMQQC DSPHVVKYYG SYFKNTDLWI VMEYCGAGSV SDIIRLRNKT 120
121 LTEDEIATIL QSTLKGLEYL HFMRKIHRDI KAGNILLNTE GHAKLADFGV AGQLTDTMAK 180
181 RNTVIGTPFW MAPEVIQEIG YNCVADIWSL GITAIEMAEG KPPYADIHPM RAIFMIPTNP 240
241 PPTFRKPELW SDNFTDFVKQ CLVKSPEQRA TATQLLQHPF VRSAKGVSIL RDLINEAMDV 300
301 KLKRQESQQR EMDQDDEENS EEDEMDSGTM VRAVGDEMGT VRVASTMTDG ANTMIEHDDT 360
361 LPSQLGTMVI NAEDEEEEGT MKRRDETMQP AKPSFLEYFE QKEKENQINS FGKSVPGPLK 420
421 NSSDWKIPQD GDYEFLKSWT VEDLQKRLLA LDPMMEQEIE EIRQKYQSKR QPILDAIEAK 480
481 KRRQQNF                                                          540
```

blue: MST1 sequence expressed in recombinant protein **Red**: variant in recombinant protein

[1]NCBI/Protein accession number NP_006273.1
M312V: SNP variation see NCBI/dbSNP ID: rs17420378

Recombinant Proteins