Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ TSF1

serine/threonine kinase 16

Recombinant Human Active Protein Kinase

HGNC Symbol: STK16

Synonyms: FLJ39635, hPSK, KRCT, MGC16211, MPSK, MPSK1, PKL12

Product No.: 0187-0000-1

Lot: 002

Description: Human TSF1, full length, amino acids M_1-I_{305} (as in NCBI/Protein entry NP_001008910.1), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: TSF1 Lot 002, was confirmed as TSF1 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 64,393 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

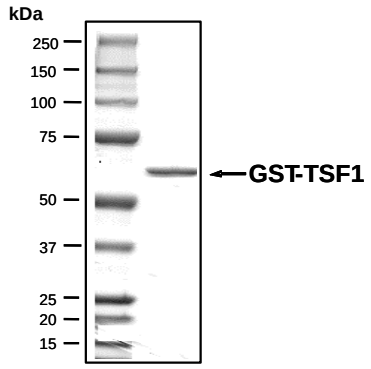
Storage buffer: 50 mM TRIS-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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

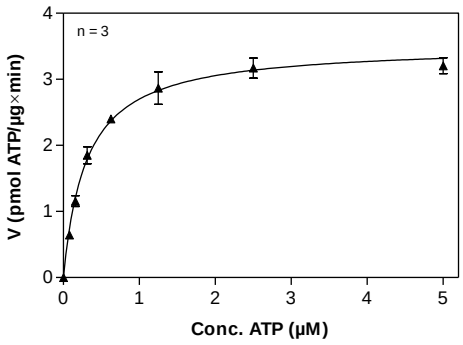
Biochemical Parameters:
Specific kinase activity (P_i transfer): 3.5 pmol/µg × min
ATP-K_M: 0.3 µM

TSF1 Lot 002:
Coomassie stain



2.0 µg GST-TSF1

TSF1 Lot 002:
Determination of V_{max} and K_M value for ATP



- 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: Casein 200 µg/ml
  Kinase: 2 µg/ml
- Filter binding assay

MSFC membrane (Millipore)

Additional assay technology:
TSF1 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

ProQinase™ TSF1

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GST-TSF1 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 GIYARGIQMG HALCVCSRGT VIIDNKRYLF IQKLGEGGFS YVDLVEGLHD 300
301 GHFYALKRIL CHEQQDREEA QREADMHRLF NHPNILRLVA YCLRERGAKH EAWLLLPFFK 360
361 RGTLWNEIER LKDKGNFLTE DQILWLLLGI CRGLEAIHAK GYAHRDLKPT NILLGDEGQP 420
421 VLMDLGSMNQ ACIHVEGSRQ ALTLQDWAAQ RCTISYRAPE LFSVQSHCVI DERTDVWSLG 480
481 CVLYAMMFGE GPYDMVFQKG DSVALAVQNQ LSIPQSPRHS SALRQLLNSM MTVDPHQRPH 540
541 IPLLLSQLEA LQPPAPGQHT TQI                                         600
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1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: TSF1

TSF1 wt[1] Amino Acid Sequence

```
  1 MGHALCVCSR GTVIIDNKRY LFIQKLGEGG FSYVDLVEGL HDGHFYALKR ILCHEQQDRE  60
 61 EAQREADMHR LFNHPNILRL VAYCLRERGA KHEAWLLLPF FKRGTLWNEI ERLKDKGNFL 120
121 TEDQILWLLL GICRGLEAIH AKGYAHRDLK PTNILLGDEG QPVLMDLGSM NQACIHVEGS 180
181 RQALTLQDWA AQRCTISYRA PELFSVQSHC VIDERTDVWS LGCVLYAMMF GEGPYDMVFQ 240
241 KGDSVALAVQ NQLSIPQSPR HSSALRQLLN SMMTVDPHQR PHIPLLLSQL EALQPPAPGQ 300
301 HTTQI                                                             360
```

blue: TSF1 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_001008910.1

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