Certificate of Analysis

REACTION BIOLOGY

ProQinase™ COT

Cancer Osaka thyroid oncogene

Recombinant Human Active Protein Kinase

HGNC Symbol: MAP3K8

Synonyms: EST; ESTF; TPL2; Tpl-2; c-COT; MEKK8

Product No.: 0180-0000-1

Lot: 018

Description: Human COT, internal fragment, amino acids M_{30}-R_{397} (as in NCBI/Protein entry NP_005195.1), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells.

Product identity: COT Lot 018, was confirmed as COT by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 71,561 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

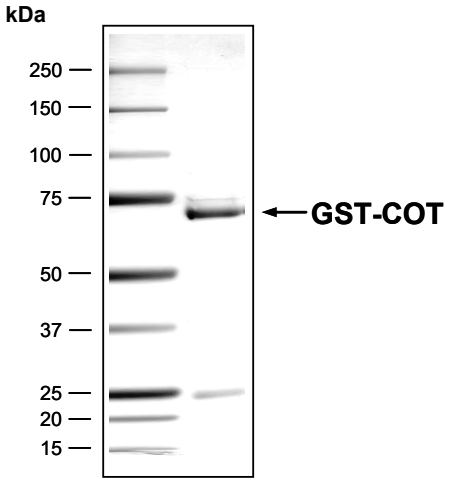
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20% glycerol

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

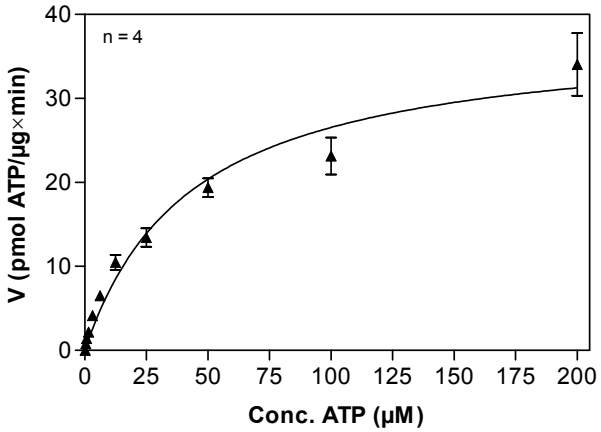
Biochemical Parameters:
Specific kinase activity (P_i transfer): 38 pmol/µg×min
ATP-K_M: 43 µM

COT Lot 018:
Coomassie stain



2.0 µg GST-COT

COT Lot 018:
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 µg/ml
  COT: 4.0 µg/ml
- Filter binding assay
  MSFC membrane (Millipore)

Recombinant Proteins

REACTION BIOLOGY

ProQinase™ COT

Product No.: 0180-0000-1

COT 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 GICSIEEFME NLYASEEPAV YEPSLMTMCQ DSNQNDERSK SLLLSGQEVP 300
301  WLSSVRYGTV EDLLAFANHI SNTAKHFYGQ RPQESGILLN MVITPQNGRY QIDSDVLLIP 360
361  WKLTYRNIGS DFIPRGAFGK VYLAQDIKTK KRMACKLIPV DQFKPSDVEI QACFRHENIA 420
421  ELYGAVLWGE TVHLFMEAGE GGSVLEKLES CGPMREFEII WVTKHVLKGL DFLHSKKVIH 480
481  HDIKPSNIVF MSTKAVLVDF GLSVQMTEDV YFPKDLRGTE IYMSPEVILC RGHSTKADIY 540
541  SLGATLIHMQ TGTPPWVKRY PRSAYPSYLY IIHKQAPPLE DIADDCSPGM RELIEASLER 600
601  NPNHRPRAAD LLKHEALNPP REDQPR                                      660
```

1-218: GST **Red:** HIS6-tag **Pink**: Thrombin cleavage site **blue**: COT fragment

COT wt[1] Amino Acid Sequence

```
  1  MEYMSTGSDN KEEIDLLIKH LNVSDVIDIM ENLYASEEPA VYEPSLMTMC QDSNQNDERS 60
 61  KSLLLSGQEV PWLSSVRYGT VEDLLAFANH ISNTAKHFYG QRPQESGILL NMVITPQNGR 120
121  YQIDSDVLLI PWKLTYRNIG SDFIPRGAFG KVYLAQDIKT KKRMACKLIP VDQFKPSDVE 180
181  IQACFRHENI AELYGAVLWG ETVHLFMEAG EGGSVLEKLE SCGPMREFEI IWVTKHVLKG 240
241  LDFLHSKKVI HHDIKPSNIV FMSTKAVLVD FGLSVQMTED VYFPKDLRGT EIYMSPEVIL 300
301  CRGHSTKADI YSLGATLIHM QTGTPPWVKR YPRSAYPSYL YIIHKQAPPL EDIADDCSPG 360
361  MRELIEASLE RNPNHRPRAA DLLKHEALNP PREDQPRCTS LDSALLERKR LLSRKELELP 420
421  ENIADSSCTG STEESEMLKR QRSLYIDLGA LAGYFNLVRG PPTLEYG               480
```

blue: COT sequence expressed in fusionprotein

[1]NCBI/Protein accession number NP_005195.1

Recombinant Proteins