

Certificate of Analysis



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 M₃₀-R₃₉₇ (as in NCBI/Protein entry NP_005195.1), N-terminal GST-HIS₆ 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!

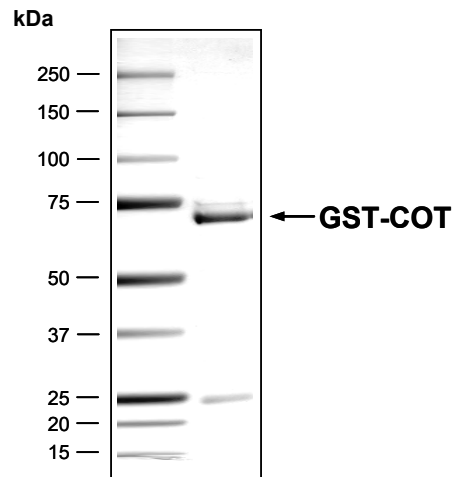
Protein concentration: 0.088 µg/µl

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

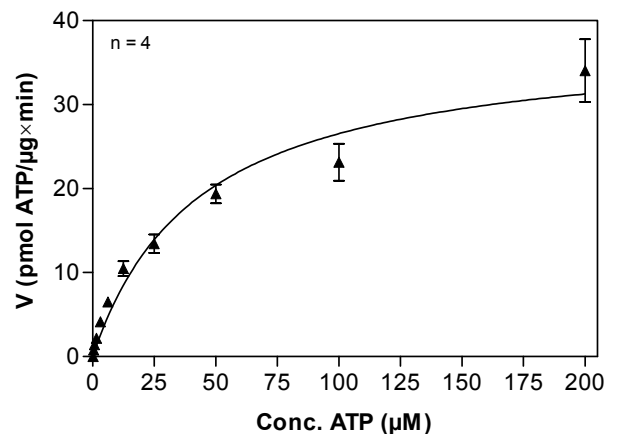
Biochemical Parameters:

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

COT Lot 018: Coomassie stain



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 MgCl₂
 - 3 mM MnCl₂
 - 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)

COT

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COT Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSM	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIQID	KYLKSSKYIA	WPLQGQWATF	GGGDHPPKSD	PMGHHHHHGG	RRRASVAAGI	240
241	LVPRGSPGLD	GICSIEEFME	NLYASEEPAV	YEPSLMTMCQ	DSNQNDERSK	SLLLSGQEV	300
301	WLSSVRYGTV	EDLLAFANHI	SNTAKHFGYQ	RPQESGILLN	MVITPQNGRY	QIDSDVLLIP	360
361	WKLYRNI	DFIPRGAFGK	VYLAQDIKTK	KRMACKLIPV	DQFKPSDVEI	QACFRHENIA	420
421	ELYGAVLWGE	TVHLFMEAGE	GGSVLEKLES	CGPMREFEII	WVTKHVLKGL	DFLHKKVIH	480
481	HDIKPSNIVF	MSTKAVLVDF	GLSVQMTEDV	YFPKDLRGTE	IYMSPEVILC	RGHSTKADIY	540
541	SLGATLIHM	TGTPPWVKRY	PRSAYPYLY	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	ISNTAKHFGY	QRQESGILL	NMVITPQNGR	120
121	YQIDSDVLLI	PWKLTYRNIG	SDFIPRGAFG	KVYLAQDIKT	KRMACKLIP	VDQFKPSDVE	180
181	IQACFRHENI	AELYGAVLWG	ETVHLFMEAG	EGGSVLEKLE	SCGPMREFEII	IWVTKHVLKG	240
241	LDFLHKKVI	HHDIKPSNIV	FMSTKAVLVD	FGLSVQMTED	VYFPKDLRGTE	EIYMSPEVIL	300
301	CRGHSTKADI	YSLGATLIHM	QTGTPPWVKR	YPRSAYPYLY	YIIHKQAPPL	EDIADDCSPG	360
361	MRELIEASLE	RNPNHRPRAA	DLLKHEALNP	PREDQPRCTS	LDSALLERKR	LLSRKELELP	420
421	ENIADSSCTG	STEESEMLKR	QRSLYIDLGA	LAGEYFNLV	RG	PPTLEYG	480

blue: COT sequence expressed in fusionprotein

¹NCBI/Protein accession number NP_005195.1