Certificate of Analysis

REACTION BIOLOGY

ProQinase™ MKK3

mitogen-activated protein kinase kinase 3

Recombinant Human Active Protein Kinase

HGNC Symbol: MAP2K3

Synonyms: MEK3; MAPKK3; SAPKK2

Product No.: 1965-0000-1

Lot: 001

Description: Human MKK3, full length, amino acids M_1-S_{347} (as in NCBI/Protein entry NP_659731.1), N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: MKK3 Lot 001, has been verified by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 69520 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: With MEKK3

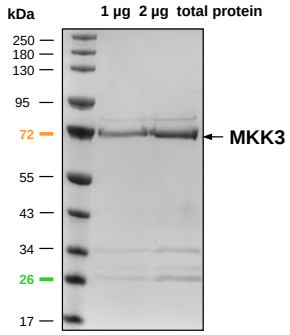
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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!

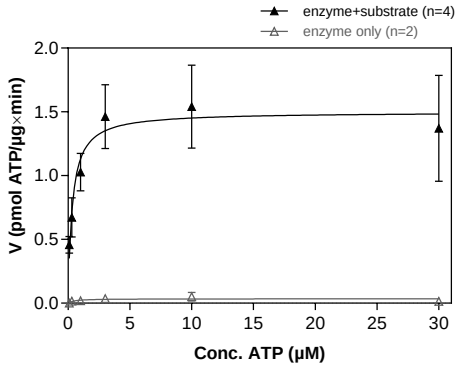
Protein concentration: 0.263 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 1.5 pmol/µg*min
ATP-K_M: 0.33 µM

MKK3 Lot 001:
Coomassie stain



MKK3 Lot 001:
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: p38alpha K53A 40 µg/ml
  Kinase: 4 µg/ml

Assay technology:
Radiometric filter binding assay
MSFC membrane (96 well plate, Millipore)

Recombinant Proteins

Sequence information

GST-MKK3 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 KMFKDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
181  KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RDSLEVLFQG 240
241  PMESPASSQP ASMPQSKGKS KRKKDLRISC MSKPPAPNPT PPRNLDSRTF ITIGDRNFEV 300
301  EADDLVTISE LGRGAYGVVE KVRHAQSGTI MAVKRIRATV NSQEQKRLLM DLDINMRTVD 360
361  CFYTVTFYGA LFREGDVWIC MELMDTSLDK FYRKVLDKNM TIPEDILGEI AVSIVRALEH 420
421  LHSKLSVIHR DVKPSNVLIN KEGHVKMCDF GISGYLVDSV AKTMDAGCKP YMAPERINPE 480
481  LNQKGYNVKS DVWSLGITMI EMAILRFPYE SWGTPFQQLK QVVEEPSPQL PADRFSPEFV 540
541  DFTAQCLRKN PAERMSYLEL MEHPFFTLHK TKKTDIAAFV KEILGEDSPW ELAAACRVPP 600
601  GDLYRLC                                                           660
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1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: MKK3

MKK3 wt[1] Amino Acid Sequence

```
  1  MESPASSQPA SMPQSKGKSK RKKDLRISCM SKPPAPNPTP PRNLDSRTFI TIGDRNFEVE  60
 61  ADDLVTISEL GRGAYGVVEK VRHAQSGTIM AVKRIRATVN SQEQKRLLMD LDINMRTVDC 120
121  FYTVTFYGAL FREGDVWICM ELMDTSLDKF YRKVLDKNMT IPEDILGEIA VSIVRALEHL 180
181  HSKLSVIHRD VKPSNVLINK EGHVKMCDFG ISGYLVDSVA KTMDAGCKPY MAPERINPEL 240
241  NQKGYNVKSD VWSLGITMIE MAILRFPYES WGTPFQQLKQ VVEEPSPQLP ADRFSPEFVD 300
301  FTAQCLRKNP AERMSYLELM EHPFFTLHKT KKTDIAAFVK EILGEDS               360
```

blue: MKK3 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_659731.1

Recombinant Proteins