Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ MKK7

mitogen-activated protein kinase kinase 7

Recombinant Human Active Protein Kinase

HGNC Symbol: MAP2K7

Synonyms: MAPKK7, MEK7, PRKMK7

Product No.: 0726-0000-1

Lot: 004

Description: Human MKK7, full length, amino acids M_1-R_{419} (as in NCBI/Protein entry NP_660186.1), N-terminal HIS_6 fusion protein with a Thrombin and TEV cleavage site, expressed in Sf9 insect cells

Product identity: MKK7 Lot 002, was confirmed as MKK7 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 52,809 Da Da

Expression host: Sf9 insect cells

Purification: Immobilized Metal Affinity Chromatography

Activation: With MEKK3

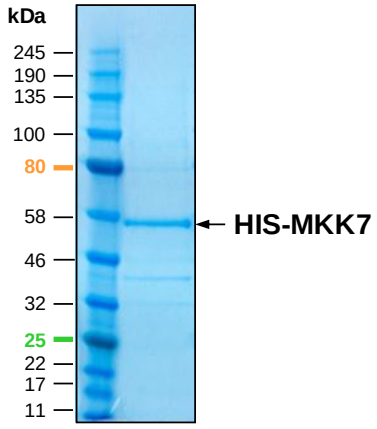
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 20 % glycerol

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

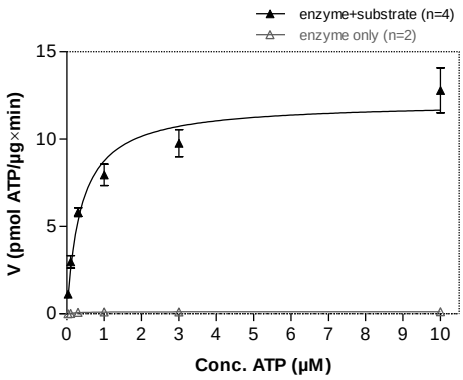
Biochemical Parameters:
Specific kinase activity (P_i transfer): 12 pmol/µg × min
ATP-K_M: 0.4 µM

MKK7 Lot 004:
Coomassie stain



1 µg HIS-MKK7 LOT004

MKK7 Lot 004:
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:JNK1 K55R/K56R 40 µg/ml
Kinase: 2 µg/ml
• Filter binding assay
MSFC membrane (Millipore)

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HIS-MKK7 Recombinant Fusion Protein Amino Acid Sequence

```
1    MSPIDPMGHH HHHHGRRRAS VAAGILVPRG SPGLDGIYAR TENLYFQGAM AASSLEQKLS  60
61   RLEAKLKQEN REARRRIDLN LDISPQRPRP TLQLPLANDG GSRSPSSESS PQHPTPPARP  120
121  RHMLGLPSTL FTPRSMESIE IDQKLQEIMK QTGYLTIGGQ RYQAEINDLE NLGEMGSGTC  180
181  GQVWKMRFRK TGHVIAVKQM RRSGNKEENK RILMDLDVVL KSHDCPYIVQ CFGTFITNTD  240
241  VFIAMELMGT CAEKLKKRMQ GPIPERILGK MTVAIVKALY YLKEKHGVIH RDVKPSNILL  300
301  DERGQIKLCD FGISGRLVDS KAKTRSAGCA AYMAPERIDP PDPTKPDYDI RADVWSLGIS  360
361  LVELATGQFP YKNCKTDFEV LTKVLQEEPP LLPGHMGFSG DFQSFVKDCL TKDHRKRPKY  420
421  NKLLEHSFIK RYETLEVDVA SWFKDVMAKT ESPRTSGVLS QPHLPFFR              480
```

Red: HIS6-tag **Pink**: Thrombin cleavage site **Green**: TEV cleavage site **blue**: MKK7

MKK7 wt[1] Amino Acid Sequence

```
  1  MAASSLEQKL SRLEAKLKQE NREARRRIDL NLDISPQRPR PTLQLPLAND GGSRSPSSES  60
 61  SPQHPTPPAR PRHMLGLPST LFTPRSMESI EIDQKLQEIM KQTGYLTIGG QRYQAEINDL  120
121  ENLGEMGSGT CGQVWKMRFR KTGHVIAVKQ MRRSGNKEEN KRILMDLDVV LKSHDCPYIV  180
181  QCFGTFITNT DVFIAMELMG TCAEKLKKRM QGPIPERILG KMTVAIVKAL YYLKEKHGVI  240
241  HRDVKPSNIL LDERGQIKLC DFGISGRLVD SKAKTRSAGC AAYMAPERID PPDPTKPDYD  300
301  IRADVWSLGI SLVELATGQF PYKNCKTDFE VLTKVLQEEP PLLPGHMGFS GDFQSFVKDC  360
361  LTKDHRKRPK YNKLLEHSFI KRYETLEVDV ASWFKDVMAK TESPRTSGVL SQPHLPFFR   420
```

blue: MKK7 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_660186.1

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