Certificate of Analysis

ProQinase TARGETING CANCER

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

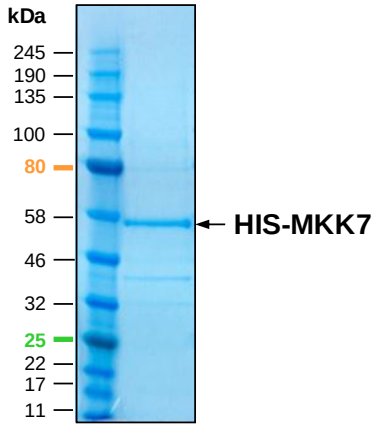
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 20 % glycerol

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)

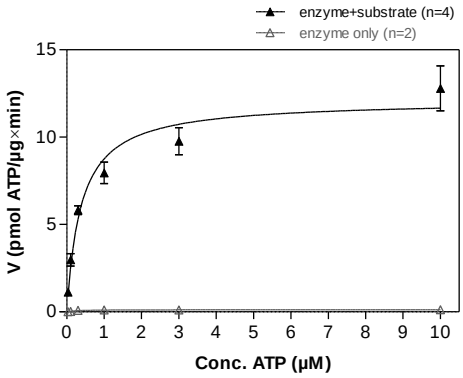
Biochemical Parameters:
Specific kinase activity (P_i transfer): 12 pmol/µg × min
ATP-K_M: 0.4 µM

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)

Recombinant Proteins

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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

Recombinant Proteins