

MKK4

mitogen-activated protein kinase kinase 4

Recombinant Human Active Protein Kinase

HGNC Symbol: MAP2K4

Synonyms: JNKK, JNKK1, MAPKK4, MEK4, PRKMK4, SEK1, SERK1

Product No.: 0948-0000-1

Lot: 001

Description: Human MKK4, full length, amino acids M₁-D₃₉₉ (as in [NCBI/Protein](#) entry NP_003001.1), activated, N-terminal HIS₆ fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: MKK4 Lot 001, was confirmed as MKK4 by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 49,113 Da

Expression host: Sf9 insect cells

Purification: Immobilized Metal Affinity Chromatography

Activation: With MEKK2

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: 0.884 µg/µl

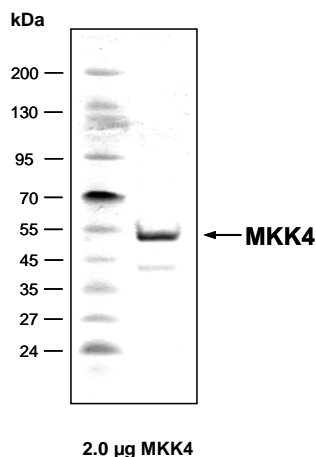
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

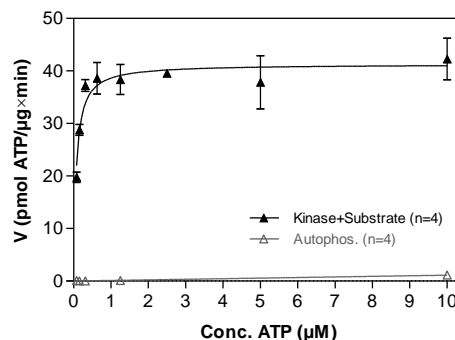
Specific kinase activity (P_i transfer): 41 pmol/µg × min

ATP-K_M: 0.07 µM

MKK4 Lot 001: Coomassie stain



MKK4 Lot 001: 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: JNK1 K55R/K56R, 60 µg/ml
 - MKK4: 0.5 µg/ml
- Radiometric Filter binding assay
- MSFC membrane (Millipore)

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HIS-MKK4 Recombinant Fusion Protein Amino Acid Sequence												
1	MSPIDPMG	HH	HHH	GRRRAS	VAAGI	LVPRG	SPGLDGIYAR	GIQAS	MAAPS	PSGGGGSGGG	60	
61	RGSGT	PGPVG	SPAPG	HPAVS	SMQGK	RKALK	LNLFAN	PPFKS	TARFTL	NPNP	TGVQNP	120
121	LRTHS	IESSG	KLKIS	PEQHW	DFTAED	LKDL	GEIGR	GAYGS	VNKMV	HKPSG	QIMAVK	180
181	TVDEK	EQKQL	IMDL	DVVMRS	SDCPY	IYQFY	GALFR	EGDCW	ICMEL	MSTSF	DKFYKY	240
241	LDDVI	PEEIL	GKITL	ATVKA	LNHLK	ENLKI	IHRDI	PSNI	LLDRS	GNIKL	CDFGIS	300
301	DSIAK	TRDAG	CRPYM	APERI	DPSAS	RQGYD	VRSDV	WLSGI	TLYEL	ATGRE	PYPKWN	360
361	QLTQV	VKQDP	PQLSN	SEERE	FSPSF	INFLC	TKDES	KR	PKYK	ELLKH	FILMYE	420
421	EVACY	VCKIL	DQMPAT	PSSP	MYVD							480

Red: HIS6-tag Pink: Thrombin cleavage site blue: MKK4

MKK4 wt ¹ Amino Acid Sequence													
1	MAAPS	PSGGG	GSGGG	SGSGT	PGPVG	SPAPG	HPAVS	SMQK	RKALK	LNLFAN	PPFK	STARFT	60
61	LNPNT	TGVQN	PHIER	LRTHS	IESSG	KLKIS	PEQHW	DFTAE	DLKDL	GEIGR	GAYGS	VNKMV	120
121	HKPSG	QIMAV	KRIRST	VDEK	EQQLL	MDLD	VMRSS	DCPY	IVQFY	GALFR	EGDC	WICMEL	180
181	MSTSF	DKFYK	YVYV	LDDVI	PEEIL	GKITL	ATVKA	LNHLK	ENLKI	IHRDI	KPSN	ILLDRS	240
241	GNIKL	CDFGI	SGQLV	DSIAK	TRDAG	CRPYM	APERI	DPSAS	RQGYD	VRSDV	WLSGI	TLYEL	300
301	ATGRF	PYPKW	NSVFD	QLTQV	VKQDP	PQLSN	SEERE	FSPSF	INFLC	TKDES	KR	PKYKE	360
361	LLKHP	FILMY	EERAVE	VACY	VCKIL	DQMPA	TPSSP	MYVD					420

blue: MKK4 sequence expressed in recombinant protein

¹[NCBI/Protein](#) accession number NP_003001.1

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