

Certificate of Analysis



MKK6 S207D/T211D

mitogen-activated protein kinase kinase 6

Recombinant Human Active Protein Kinase

HGNC Symbol: MAP2K6

Synonyms: MAPKK6; MEK6; MKK6; PRKMK6; SAPKK3

Product No.: 0396-0000-1

Lot: 001

Description: Human MKK6 S207D/T211D, full length, amino acids M₁-D₃₃₄ (as in NCBI/Protein entry NP_002749.2), mutationally activated: S207D/T211D N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: MKK6 S207D/T211D Lot 001, was confirmed as MKK6 by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 67,467 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

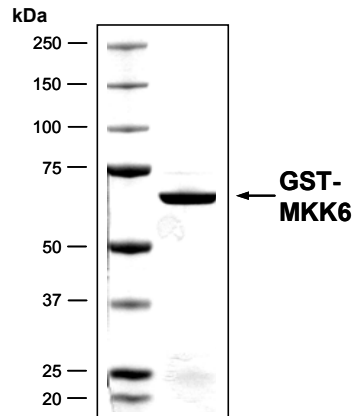
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20% glycerol

Storage temperature: -80°C
Avoid repeated freeze-thaw cycles!

Protein concentration: 1.150 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

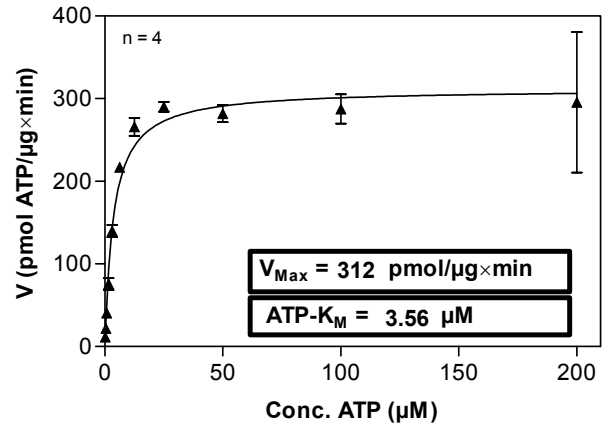
Biochemical Parameters:
Specific activity: 312 pmol/µg×min
ATP-K_M: 3,6 µM

MKK6 S207D/T211D Lot 001: Coomassie stain



2.0 µg GST-MKK6

MKK6 S207D/T211D 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:p38-alpha K53A, 100 µg / ml
 - MKK6 S207D/T211D: 4.0 µg / ml
- Filter binding assay
 - MSFC membrane (Millipore)

Additional assay technology: MKK6 S207D/T211D Lot 001

was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega

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MKK6 S207D/T211D

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GST-MKK6 S207D/T211D Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGQWQATF	GGGDHPPKSD	PMGHHHHHHG	RRRASVAAGI	240
241	LVPRGSPGLD	GICSRNSGLH	GMSQSKGKKR	NPGLKIPKEA	FEQPQTSSTP	PRDLDSKACI	300
301	SIGNQNFVEK	ADDLEPIMEL	GRGAYGVVEK	MRHVPSGQIM	AVKRIRATVN	SQEQKRLIMD	360
361	LDISMRTVDC	PFTATFYGAL	FREGDVWICM	ELMDTSLDKF	YKQVIDKGQT	IPEDILGKIA	420
421	VSIVKALEHL	HSKLSVIHRD	VKPSNVLINA	LGQVKMCDFG	ISGYLVDVA	KIDAGCKPY	480
481	MAPERINPEL	NQKGYSVKSD	IWSLGITMIE	LAILRFPYDS	WGTPFQQLKQ	VVEEPSQLP	540
541	ADKFSAEFVD	FTSQCLKNS	KERPTYPELM	QHPFFTLHES	KGTDVASFVK	LILGD	600

1-218: GST RED: HIS6-tag PINK: Thrombin blue: MKK6 A/D/D: variant aminoacids

MKK6 wt ¹ amino acid sequence							
1	MSQSKGKKRN	PGLKIPKEAF	EQPQTSSTPP	RDLDSKACIS	IGNQNFVEKA	DDLEPIMELG	60
61	RGAYGVVEKM	RHVPSGQIMA	VKRIRATVNS	QEQKRLIMDL	DISMRTVDCP	FTVTFYGALF	120
121	REGDVWICME	LMDTSLDKFY	KQVIDKGQTI	PEDILGKIAV	SIVKALEHLH	SKLSVIHRDV	180
181	KPSNVLINAL	GQVKMCDFGI	SGYLVDVSAK	TIDAGCKPYM	APERINPELN	QKGYSVKSDI	240
241	WSLGITMIEL	AILRFPYDSW	GTPFQQLKQV	VEEPSQLPA	DKFSAEFVDF	TSQCLKKNK	300
301	ERPTYPELMQ	HPFFTLHESK	GTDVASFVKL	ILGD			360

blue: MKK6 sequence expressed in fusionprotein Red: variant in fusionprotein

¹NCBI/Protein accession number NP_002749.2