Certificate of Analysis

REACTION BIOLOGY

ProQinase™ JNK1 K55R/K56R

mitogen-activated protein kinase 8

Recombinant Protein Kinase Substrate

HGNC Symbol: MAPK8

Synonyms: PRKM8, SAPK1

Product No.: 0524-0000-1

Lot: 006

Description: Human JNK1, full length, amino acids M_1-Q_{384} (as in NCBI/Protein entry NP_002741.1), inactivated by K55R and K56R mutations, N-terminal HIS_6 fusion protein with a TEV cleavage site, expressed in E.coli

Theoretical $MW_{Fusion\ Protein}$: 46,466 Da

Expression host: E.coli

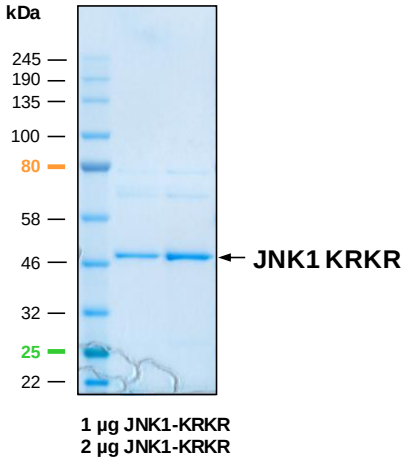
Purification: Immobilized Metal Affinity Chromatography

Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 1 mM DTT, 10% 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!

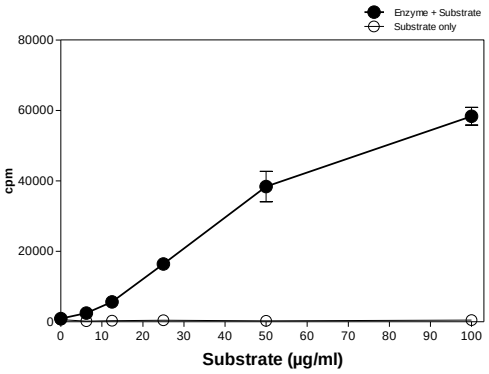
Protein concentration: 1.758 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Coomassie stain



1 µg JNK1-KRKR
2 µg JNK1-KRKR

Phosphorylation of JNK1 K55R/K56R by the kinase MKK4 (Radiometric filter binding assay):



Assay conditions:
70 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: 1 µM
Substrate: variable concentration
Kinase: 1 µg/ml
MSFC membrane (Millipore)

Field of application:
JNK1 K55R/K56R has been validated for use in radiometric in-vitro kinase activity assays. It was not validated for use in ATP-consumption based kinase activity assays.

Recombinant Proteins

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ProQinase™ JNK1 K55R/K56R

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JNK1 K55R/K56R Recombinant Fusion Protein Amino Acid Sequence

1	MHHHHHHAMT ENLYFQGAMS RSKRDNNFYS VEIGDSTFTV LKRYQNLKPI GSGAQGIVCA	60
61	AYDAILERNV AIRRLSRPFQ NQTHAKRAYR ELVLMKCVNH KNIIGLLNVF TPQKSLEEFQ	120
121	DVYIVMELMD ANLCQVIQME LDHERMSYLL YQMLCGIKHL HSAGIIHRDL KPSNIVVKSD	180
181	CTLKILDFGL ARTAGTSFMM TPYVVTRYYR APEVILGMGY KENVDLWSVG CIMGEMVCHK	240
241	ILFPGRDYID QWNKVIEQLG TPCPEFMKKL QPTVRTYVEN RPKYAGYSFE KLFPDVLFPA	300
301	DSEHNKLKAS QARDLLSKML VIDASKRISV DEALQHPYIN VWYDPSEAEA PPPKIPDKQL	360
361	DEREHTIEEW KELIYKEVMD LEERTKNGVI RGQPSPLAQV QQ	420

Red: 6HIS-tag **Green**: TEV site **blue**:JNK1 K55R/K56R **boxed**: K55R/K56R point mutations

JNK1 wt1 Amino Acid Sequence

1	MSRSKRDNNF YSVEIGDSTF TVLKRYQNLK PIGSGAQGIV CAAYDAILER NVAIKKLSRP	60
61	FQNQTHAKRA YRELVLMKCV NHKNIIGLLN VFTPQKSLEE FQDVYIVMEL MDANLCQVIQ	120
121	MELDHERMSY LLYQMLCGIK HLHSAGIIHR DLKPSNIVVK SDCTLKILDF GLARTAGTSF	180
181	MMTPYVVTRY YRAPEVILGM GYKENVDLWS VGCIMGEMVC HKILFPGRDY IDQWNKVIEQ	240
241	LGTPCPEFMK KLQPTVRTYV ENRPKYAGYS FEKLFPDVLF PADSEHNKLK ASQARDLLSK	300
301	MLVIDASKRI SVDEALQHPY INVWYDPSEA EAPPPKIPDK QLDEREHTIE EWKELIYKEV	360
361	MDLEERTKNG VIRGQPSPLA QVQQ	420

blue: JNK1 sequence expressed in fusionprotein **Red**: variant in fusionprotein

[1]NCBI/Protein accession number NP_002741.1

Recombinant Proteins