

# Certificate of Analysis



## JNK1 K55R/K56R

c-Jun N-terminal kinase 1

Recombinant Protein Kinase Substrate

HGNC Symbol: MAPK8

Synonyms: PRKM8, SAPK1

Product No.: 0524-0000-1

Lot: 005

**Description:** Human JNK1, full length, amino acids M<sub>1</sub>-Q<sub>384</sub> (as in NCBI/Protein entry NP\_002741.1), inactivated by K55R and K56R mutations, N-terminal HIS<sub>6</sub> fusion protein with a TEV cleavage site, expressed in E.coli

**Theoretical MW**<sub>Fusion Protein</sub>: 46,466 Da

**Expression:** E.coli

**Purification:** Immobilized Metal Affinity Chromatography

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

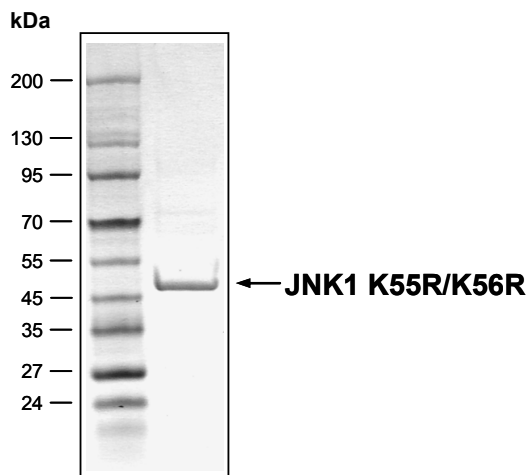
**Storage temperature:** -80°C

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:** 0.410 µg/µl

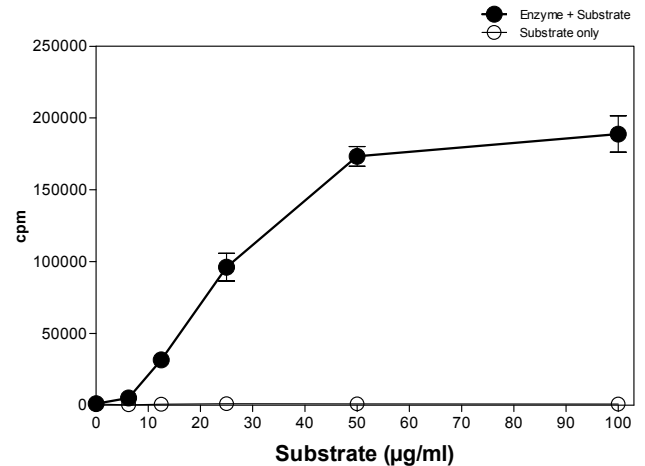
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

**Coomassie stain:**



2.0 µg JNK1 K55R/K56R

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



Assay mixture:

70 mM HEPES-NaOH, pH 7.5

3 mM MgCl<sub>2</sub>

3 mM MnCl<sub>2</sub>

3 µM Na-orthovanadate

1.2 mM DTT

50 µg/ml PEG<sub>20,000</sub>

ATP: 1 µM

Substrate (JNK1 K55R/K56R): variable concentration

MKK4: 2.0 µg/ml

MSFC membrane (Millipore)

**Field of application:**

JNK1 K55R/K56R has been validated for use in radiometric in-vitro kinase activity assays. It is not successfully validated for use in ATP-consumption based kinase activity assays.

## JNK1 K55R/K56R

Product No.: 0524-0000-1

JNK1 K55R/K56R Recombinant Fusion Protein Amino Acid Sequence							
1	MHHHHH	AMT	ENLYFQG	AMS	RSKRDNNFYS	VEIGDSTFTV LKRYQNLKPI GSGAQGIVCA	60
61	AYDAILERNV	AIRRLSRPFQ	NQTHAKRAYR	ELVLMKCVNH	KNIIGLLNVF	TPQKSLEEFQ	120
121	DVYIVMELMD	ANLCQVIQME	LDHERMSYLL	YQMLCGIKHL	HSAGIIHRDL	KPSNIVVKSD	180
181	CTLKILDFGL	ARTAGTSFMM	TPYVVTRYR	APEVILGMGY	KENVDLWSVG	CIMGEMVCHK	240
241	ILFPGRDYID	QWNKVIEQLG	TPCPEFMKKL	QPTVRTYVEN	RPKYAGYSFE	KLFPDVLFFA	300
301	DSEHNKLKAS	QARDLLSKML	VIDASKRISV	DEALQHPYIN	VWYDPSEAEA	PPPKIPDKQL	360
361	DEREHTIEEW	KELIYKEVMD	LEERTKNGVI	RGQPSPLAQV	QQ		420

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

JNK1 wt <sup>1</sup> Amino Acid Sequence							
1	MSRSKRDNNF	YSVEIGDSTF	TVLKRYQNLK	PIGSGAQGIV	CAAYDAILER	NVAIKKLSRP	60
61	FQNQTHAKRA	YRELVLMKCV	NHKNIIGLLN	VFTPKSLEE	FQDVYIVMEL	MDANLCQVIQ	120
121	MELDHERMSY	LLYQMLCGIK	HLHSAGIIHR	DLKPSNIVVK	SDCTLKILDF	GLARTAGTSF	180
181	MMTPYVVTRY	YRAPEVILGM	GYKENVDLWS	VGCIMGEMVC	HKILFPGRDY	IDQWNKVIEQ	240
241	LGTPCPEFMK	KLQPTVRTYV	ENRPKYAGYS	FEKLPDVLV	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

<sup>1</sup>NCBI/Protein accession number NP\_002741.1