Certificate of Analysis

REACTION BIOLOGY

ProQinase™ MEK1 P124L

mitogen-activated protein kinase 1

Recombinant Human Active Protein Kinase

HGNC Symbol: MAP2K1

Synonyms: MKK1, MAPKK1

Product No.: 1918-0000-1

Lot: 001

Description: Human MEK1, full length, amino acids M_1-V_{393} (as in NCBI/Protein entry NP_002746.1), with a P124L point mutation, activated, N-terminal HIS-tag, expressed in Sf9 insect cells

Product identity: MEK1 P124L Lot 001, was confirmed as MEK1 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 46576 Da

Expression host: Sf9 insect cells

Purification: Immobilized Metal Affinity Chromatography

Activation: with RAF1

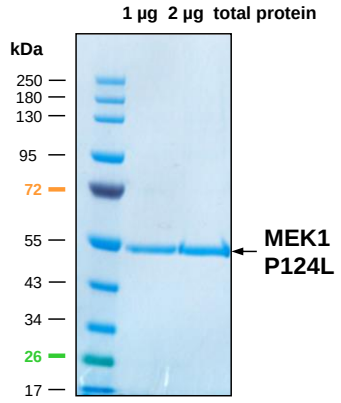
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 20 % glycerol

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!

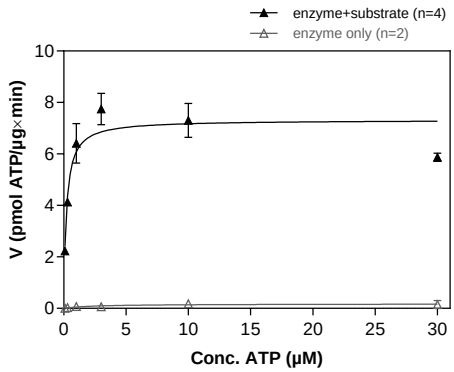
Protein concentration: 0.424 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 7.3 pmol/µg x min
ATP-K_M: 0.2 µM

MEK1 P124L Lot 001:
Coomassie stain



MEK1 P124L Lot 001:
Determination of V_{max} and K_M value for ATP



- 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: ERK2 K54R 100 µg/ml
  Kinase: 1 µg/ml
- Filter binding assay

MSFC membrane (Millipore)

Recombinant Proteins

Sequence information

HIS-MEK1 P124L Recombinant Fusion Protein Amino Acid Sequence

```
  1 MSPILGDPMG HHHHHHGRDS LEVLFQGPMP KKKPTPIQLN PAPDGSAVNG TSSAETNLEA  60
 61 LQKKLEELEL DEQQRKRLEA FLTQKQKVGE LKDDDFEKIS ELGAGNGGVV FKVSHKPSGL 120
121 VMARKLIHLE IKPAIRNQII RELQVLHECN SLYIVGFYGA FYSDGEISIC MEHMDGGSLD 180
181 QVLKKAGRIP EQILGKVSIA VIKGLTYLRE KHKIMHRDVK PSNILVNSRG EIKLCDFGVS 240
241 GQLIDSMANS FVGTRSYMSP ERLQGTHYSV QSDIWSMGLS LVEMAVGRYP IPPPDAKELE 300
301 LMFGCQVEGD AAETPPRPRT PGRPLSSYGM DSRPPMAIFE LLDYIVNEPP PKLPSGVFSL 360
361 EFQDFVNKCL IKNPAERADL KQLMVHAFIK RSDAEEVDFA GWLCSTIGLN QPSTPTHAAG 420
421 V                                                               480
```

Red: HIS6-tag **Green:** 3C cleavage site **blue**: MEK1 **boxed**: P124L mutation

MEK1 wt[1] Amino Acid Sequence

```
  1 MPKKKPTPIQ LNPAPDGSAV NGTSSAETNL EALQKKLEEL ELDEQQRKRL EAFLTQKQKV  60
 61 GELKDDDFEK ISELGAGNGG VVFKVSHKPS GLVMARKLIH LEIKPAIRNQ IIRELQVLHE 120
121 CNSPYIVGFY GAFYSDGEIS ICMEHMDGGS LDQVLKKAGR IPEQILGKVS IAVIKGLTYL 180
181 REKHKIMHRD VKPSNILVNS RGEIKLCDFG VSGQLIDSMA NSFVGTRSYM SPERLQGTHY 240
241 SVQSDIWSMG LSLVEMAVGR YPIPPPDAKE LELMFGCQVE GDAAETPPRP RTPGRPLSSY 300
301 GMDSRPPMAI FELLDYIVNE PPPKLPSGVF SLEFQDFVNK CLIKNPAERA DLKQLMVHAF 360
361 IKRSDAEEVD FAGWLCSTIG LNQPSTPTHA AGV                             420
```

blue: kinase sequence expressed in recombinant protein **Red**: variant in recombinant protein

[1]NCBI/Protein accession number NP_002746.1

Recombinant Proteins