Certificate of Analysis

REACTION BIOLOGY

ProQinase™ MEK1 K97M

mitogen-activated protein kinase kinase 1

Recombinant Proteins

Recombinant Protein Kinase Substrate

HGNC Symbol: MAP2K1

Synonyms: PRKMK1, MAPKK1, MKK1

Product No.: 0785-0000-1

Lot: 070

Description: Human MEK1, full length, amino acids M_1-V_{393} (as in NCBI/Protein entry NP_002746.1), mutationally inactivated by amino acid exchange $K_{97}M$, untagged, expressed in Sf9 insect cells/E.coli

Theoretical $MW_{Fusion\ Protein}$: 43,784 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

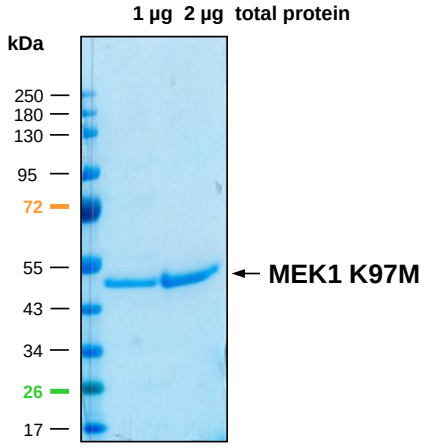
ATPase activity: In an ADP-Glo™ assay (Promega), the ATP → ADP conversion within 30 min is approx. 1% at a concentration of 100 µg/ml substrate.
Detailed ATPase assay conditions on request

Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 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!

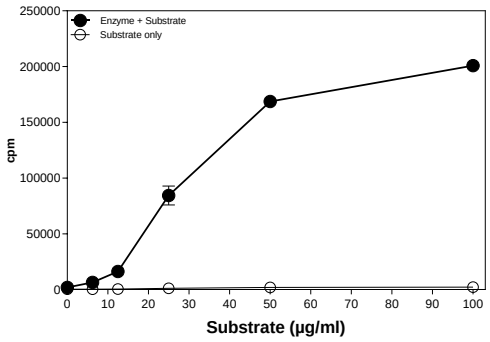
Protein concentration: 0.464 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Substrate Lot 070:
Coomassie stain



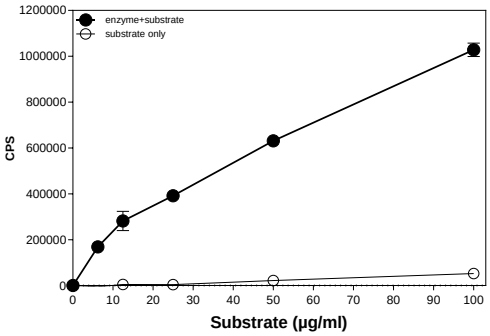
Phosphorylation of SUBSTRATE by KINASE

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 filter plates (Corning)

ADP-Glo™ assay (Promega)



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 PEG20.000
ATP: variable concentration
1 % (v/v) DMSO
Substrate: 100 µg/ml
Kinase: 2 µg/ml

MEK1 K97M

Product No.: 0785-0000-1

MEK1 K97M Recombinant Fusion Protein Amino Acid Sequence

```
  1  GPLAMTKKKP TPIQLNPAPD GSAVNGTSSA ETNLEALQKK LEELELDEQQ RKRLEAFLTQ  60
 61  KQKVGELKDD DFEKISELGA GNGGVVFKVS HKPSGLVMAR MLIHLEIKPA IRNQIIRELQ 120
121  VLHECNSPYI VGFYGAFYSD GEISICMEHM DGGSLDQVLK KAGRIPEQIL GKVSIAVIKG 180
181  LTYLREKHKI MHRDVKPSNI LVNSRGEIKL CDFGVSGQLI DSMANSFVGT RSYMSPERLQ 240
241  GTHYSVQSDI WSMGLSLVEM AVGRYPIPPP DAKELELMFG CQVEGDAAET PPRPRTPGRP 300
301  LSSYGMDSRP PMAIFELLDY IVNEPPPKLP SGVFSLEFQD FVNKCLIKNP AERADLKQLM 360
361  VHAFIKRSDA EEVDFAGWLC STIGLNQPST PTHAAGV                          420
```

1-4: legacy of tag cleavage **blue**: MEK1 **boxed**: variation from RefSeq

Recombinant Proteins