

Certificate of Analysis

MEK1 K97M

MAPK / ERK activating kinase

Recombinant Protein Kinase Substrate

HGNC symbol: MAP2K1

Synonyms: MAPKK1, MAPKK 1, MKK1, PRKMK1

Product No.: 0785-0000-1

Lot: 049

Description: Human MEK1, full length, amino acids M₁-V₃₉₃ (as in NCBI/Protein entry NP_002746.1), mutationally inactivated K97M, untagged, expressed in insect cells

Theoretical MW: 43,784 Da

Expression: Baculovirus infected Sf9 cells

Purification: Affinity purification using GSH-agarose, followed by 3C mediated removal of the GST tag and size exclusion chromatography

ATPase activity: In an ADP-Glo™ assay (Promega) with 10 μM ATP or 30 μM ATP, the ATP → ADP conversion within 30 min is below 1% at a concentration of 100 μg/ml MEK1 K97M*
*detailed ATPase assay conditions on request

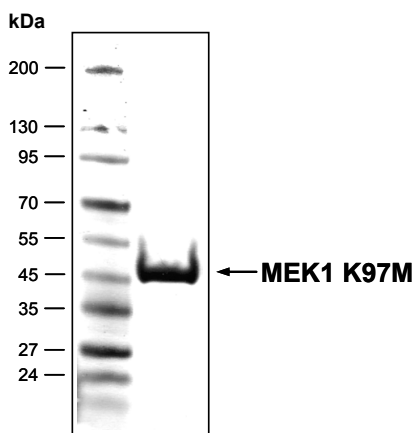
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 1 mM DTT, 10% glycerol

Storage temperature: -80°C

For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10 μl are not advisable. Avoid repeated freeze-thaw cycles!

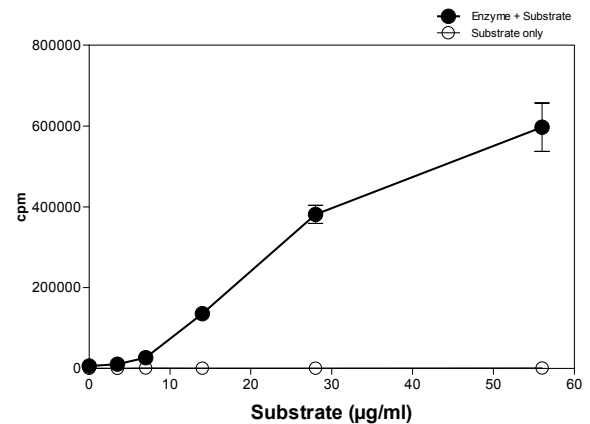
Protein concentration: 0.299 μg/μl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Coomassie stain:



3.0 μg MEK1 K97M, Lot049

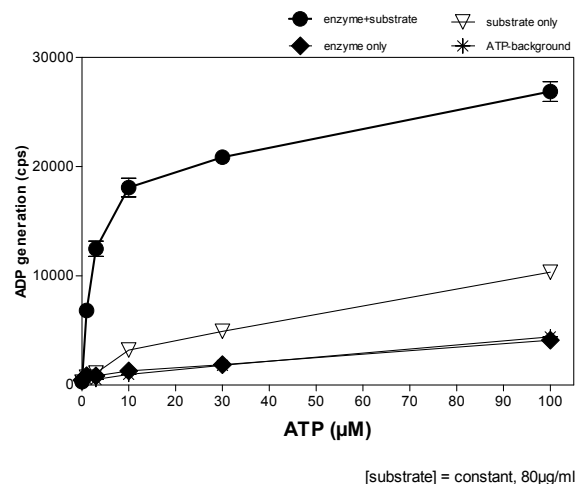
Phosphorylation of MEK1 K97M Lot049 by the kinase RAF1 Y340D/Y341D (Radiometric filter binding assay):



Assay mixture:

70 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: 1 μM
Substrate (MEK1 K97M): variable concentration
RAF1 Y340D/Y341D: 320 ng/ml
MSFC membrane (Millipore)

Phosphorylation of MEK1 K97M Lot049 by the kinase RAF1 Y340D/Y341D (ADP-Glo™ assay / Promega):



Assay mixture:

70 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 concentration
1 % (v/v) DMSO
Substrate (MEK1 K97M): 80 μg/ml
RAF1 Y340D/Y341D: 2.0 μg/ml