

ERK2 K54R (kinase-inactive)

extracellular signal regulated kinase 2

Recombinant Protein Kinase Substrate

Synonyms: ERK, MAPK2, p38

Product No.: 0416-0000-5

Lot: 011

Description: Human ERK2, full length, amino acids M₁-S₃₆₀ (as in GenBank entry NM_138957), inactivated by K54R mutation, N-terminal HIS₆ fusion protein, expressed in E.coli

Theoretical MW_{Fusion Protein}: 45,502 Da

Expression: E.coli

Purification: Affinity chromatography using Ni-MAC-agarose, followed by 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 ERK2 K54R*

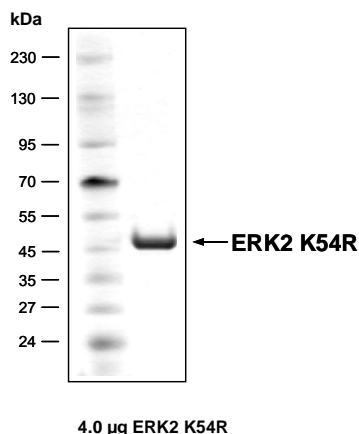
*detailed ATPase assay conditions on request

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

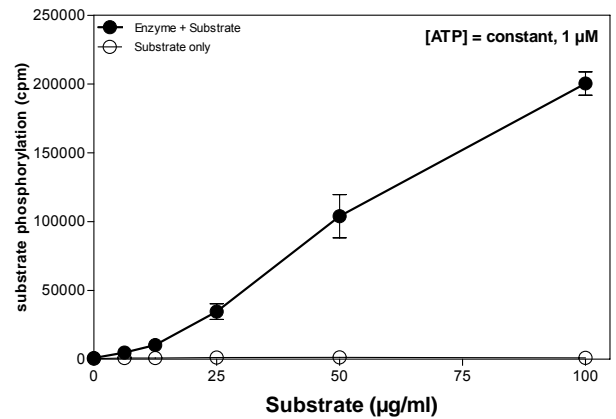
Storage temperature: -80°C
Avoid repeated freeze-thaw cycles!

Protein concentration: 1.020 μg/μl (Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Coomassie stain:



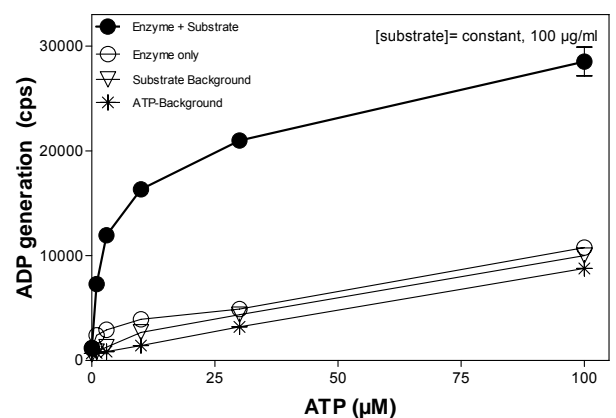
Phosphorylation of ERK2 K54R by the kinase MEK1 wt (Radiometric filter binding assay):



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 (ERK2 K54R): variable concentration
MEK1 wt: 2.0 μg/ml
MSFC membrane (Millipore)

Phosphorylation of ERK2 K54R by the kinase MEK1 wt (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 (ERK2 K54R): 100 μg/ml
MEK1 wt: 2.0 μg/ml

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