Certificate of Analysis

p38-alpha K53A (inactivated)
(mitogen-activated protein kinase 14)

Recombinant Protein Kinase Substrate

Synonyms: MAPK14, CSBP1

Product No.: 0769-0000-1

Lot: 007

Description: Human p38-alpha, full length, amino acids M_1-S_{360} (as in GenBank entry NM_001315)*, mutationally inactivated: K53A, N-terminal HIS_6 fusion protein with a TEV cleavage site, expressed in E.coli
*Sequence may contain documented polymorphisms
Detailed aa-sequence on request

Theoretical $MW_{Fusion\ Protein}$: 43,615 Da

Expression: E.coli

Purification: Immobilized Metal Affinity Chromatography followed by a size exclusion chromatography

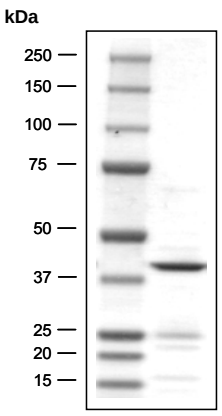
ATPase acticity: In an ADP-Glo™ assay (Promega) with 10 µM ATP or 30 µM ATP, the ATP → ADP conversion within 30 min is approx. 1% at a concentration of 100 µg/ml p38-alpha K53A*
*detailed ATPase assay conditions on request

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!

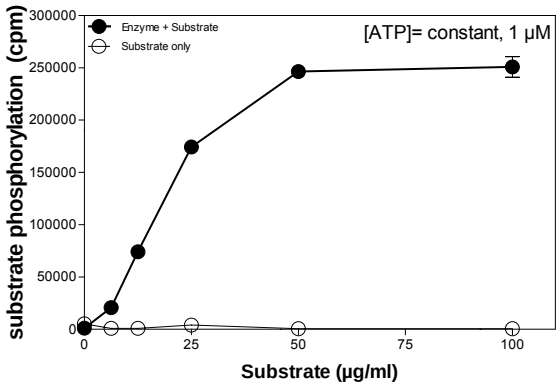
Protein concentration: 0.660 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Coomassie stain:



2 µg p38-alpha K53A

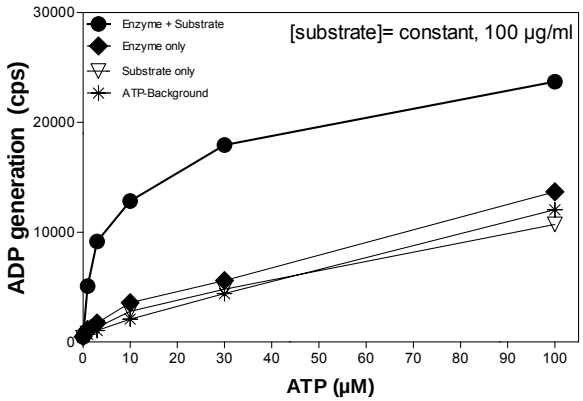
Phosphorylation of p38-alpha K53A by the kinase MKK6 S207D/T211D (Radiometric filter binding assay):



Assay mixture:

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 (p38-alpha K53A): variable concentration
MKK6 S207D/T211D: 2.0 µg/ml
MSFC membrane (Millipore)

Phosphorylation of p38-alpha K53A by the kinase MKK6 S207D/T211D (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 PEG20.000
ATP: variable concentration
1 % (v/v) DMSO
Substrate (p38-alpha K53A): 100 µg/ml
MKK6 S207D/T211D: 2.0 µg/ml

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