KinaseDetect ApS
http://www.kinasedetect.com

Technical support:
Phone: +45 66 10 66 62
e-mail:support@kinasedetect.com

Certificate of Analysis

Product	**MKK6 (MAP2K6), mutant, active** **human recombinant, expressed in E. coli, GST-fusion protein**
Cat No	PK-019-01
Lot No	151105
Description	Purified human recombinant mitogen activated protein kinase kinase 6 (MKK6), expressed in E. coli. Activated by two amino acid exchanges (S151E, T155E). Suitable for labeling MKK6 substrates. Features a gluthatione-S-transferase (GST) tag to facilitate removal of the enzyme from the reaction mixture. Sequence based calculated M.W. 63,636 Approved HUGO gene symbol: MAP2K6 Synonyms: mitogen activated protein kinase kinase 6, MEK6, SAPKK3
Quality	Protein concentration (Bradford with BSA as standard): 1.75 mg/ml Purity: > 90 % by SDS PAGE Specific activity: 162,000 Units*/ mg (* 1 Unit is defined as the amount of MKK6 which activates unactive p38alpha/SAPK2a (0.5 mg/ml) by 1 U/min using 125 µM ATP at 30 ºC. 1 U p38alpha/SAPK2a activity is defined as1 pmol phosphate transferred to myelin basic protein per min at 30 ºC) Protease activity (Twining test): none
Form	Liquid. In 50 mM Tris-HCl, 150 mM NaCl, 0.1 mM EGTA, 1 mM DTT, 270 mM sucrose, pH 7.5
Package size	20 microgram
Storage condition	-70 ^{O}C
Shipment conditions	dry ice

Material for in vitro research use only. Not for pharmaceutical or drug application. Material does not contain any animal products such as albumin.

AVOID FREEZE/THAW CYCLES

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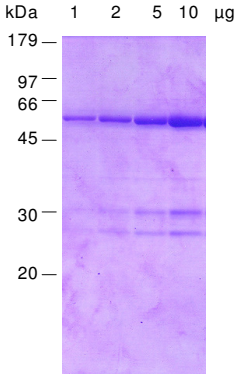
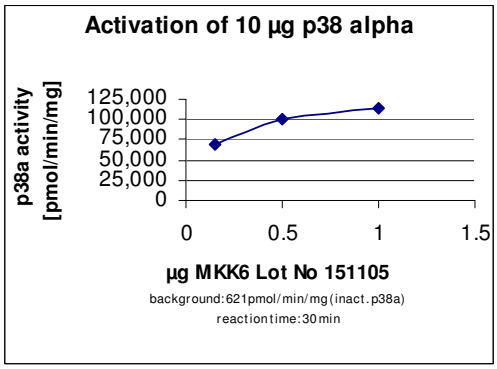
SDS-PAGE analysis



Activity determination



Amino acid sequence information

S226 corresponds to S2 of U39657, note: S431 and T435 are exchanged for E (underlined)

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MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL  50
EFPNLPYYID GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL 100
DIRYGVSRIA YSKDFETLKV DFLSKLPEML KMFEDRLCHK TYLNGDHVTH 150
PDFMLYDALD VVLYMDPMCL DAFPKLVCFK KRIEAIPQID KYLKSSKYIA 200
WPLQGWQATF GGGDHPPKSD LVPRGSQSKG KKRNPGLKIP KEAFEQPQTS 250
STPPRDLDSK ACISIGNQNF EVKADDLEPI MELGRGAYGV VEKMRHVPSG 300
QIMAVKRIRA TVNSQEQKRL LMDLDISMRT VDCPFTVTFY GALFREGDVW 350
ICMELMDTSL DKFYKQVIDK GQTIPEDILG KIAVSIVKAL EHLHSKLSVI 400
HRDVKPSNVL INALGQVKMC DFGISGYLVD EVAKEIDAGC KPYMAPERIN 450
PELNQKGYSV KSDIWSLGIT MIELAILRFP YDSWGTPFQQ LKQVVEEPSP 500
QLPADKFSAE FVDFTSQCLK KNSKERPTYP ELMQHPFFTL HESKGTDVAS 550
FVKLILGD                                              558
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Activation of p38alpha

Assay Components

One-For-All-Buffer (OFAB): 20 mM Tris-HCl, 25 mM beta-glycerol phosphate, 5 mM EGTA, 1 mM sodium orthovanadate, 1 mM DTT, pH 7.5
Protein kinase MKK6, 100 x more diluted than p38alpha/SAPK2, diluted in OFAB
Protein kinase p38alpha/SAPK2a in 50 mM Tris-HCl, 300 mM NaCl, 2 mM DTT, pH 8.5
Magnesium/ATP Cocktail**:** 75 mM $MgCl_2$, 500 microM ATP

Assay Procedure

All compounds are pipetted into a microcentrigue tube on ice
1. Add 1/8 volume OFAB
2. Add 3/8 volume H2O
3. Add 1/8 volume MKK6
4. Add 1/8 volume p38alpha/SAPK2a
5. Add 1/4 volume of Magnesium/ATP Cocktail
6. Incubate 1 h at 30 ºC.
7. Stop the reaction by setting samples on ice

The reaction might be dialyzed against 50 mM Tris-HC, 300 mM NaCl, 2 mM DTT, pH 8.5 and stored after addition of an equal volume 99 % glycerol at -70ºC.

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p38alpha/SAPK2a *in vitro* kinase assay

Assay components
One-For-All-Buffer (OFAB): 20 mM Tris-HCl, 25 mM beta-glycerol phosphate, 5 mM EGTA, 1 mM sodium orthovanadate, 1 mM DTT, pH 7.5
Substrate: myelin basic protein, 2 mg/ml
Protein kinase: p38alpha, 2.5 - 10 ng/ microliter diluted in OFAB directly before use
Magnesium/ATP Cocktail: 75 mM $MgCl_2$, 500 microM ATP
Diluted [gamma–^{32}P]ATP: Mix 197 microliter Magnesium/ATP cocktail with 3 microliter (30 µCi) [gamma–^{32}P]ATP (3,000 Ci/mmol, e.g. from Hartmann Analytic, Braunschweig)
Assay procedure
All compounds are pipetted into a microcentrifuge tube on ice
1. Add 2.5 microliter OFAB
2. Add 10 microliter myelin basic protein, 2 mg/ml
3. Add 2.5 microliter p38alpha (25 - 100 ng/assay)
4. Add 5 microliter of the diluted [gamma–^{32}P]ATP
5. Incubate 20 min at 30 ^{o}C.
6. Stop the reaction by setting samples on ice
7. Remove 10 microliter and spot on P81 paper (let bind to the paper for 30 sec)
8. Immerse the paper in 0.75% phosphoric acid, gently shake on a rotator
9. Wash 3 x with phosphoric acid
10. Wash 1 x with acetone
11. Dry under infrared light
12. Read in scintillation counter or Instant Imager

References

Chabaud-Riou M, Firestein GS (2004) Am J Pathol 164: 177-184
Zhu X, Rottkamp CA, Hartzler A, Sun Z et al. (2001) J Neurochem 79: 311-318