KinaseDetect ApS
http://www.kinasedetect.com

Technical support:
Phone: +45 66 10 66 62
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Certificate of Analysis

Product	**JNK2alpha2 (MAPK9, isoform 1), active human recombinant, expressed in E.coli, N-His-fusion protein**
Cat No.	PK-017-01
Lot No	060504
Description	Purified recombinant human JNK2alpha2 kinase, expressed in E.coli. Active form of JNK2alpha2 kinase. Suitable for labeling JNK2alpha2 kinase substrates. Features a polyhistidine tag to facilitate removal of JNK2alpha2 kinase from the reaction mixture. Purified by Ni-agarose chromatography. Sequence based calculated M.W. 49,545 Approved HUGO gene symbol: MAPK9 Synonyms: MAP kinase 9, c-Jun kinase 2, c-Jun N-terminal kinase 2, stress activated protein kinase JNK2, JNK2, Jun kinase, p54aSAPK

Quality

Protein concentration (Bradford with BSA as standard)	1.3 mg/ml
Purity	> 95% by SDS PAGE
Specific activity (* 1 Unit is defined as 1 picomole phosphate transferred to c-jun (1-169-N-His-tag) per min at 30 °C)	600 Units*/ mg
Autophosphorylation	< 5 %
Protease activity (Twining test)	none

Form	Liquid. In 50 mM Tris-HCl, 100 mM NaCl, 1 mM DTT, 50 % glycerol, pH 8.5.
Package size	100 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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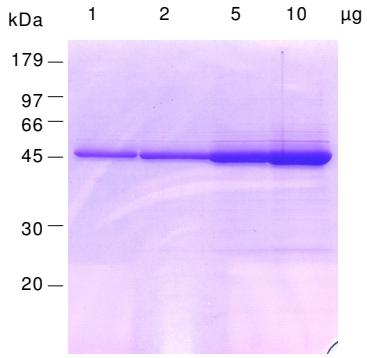
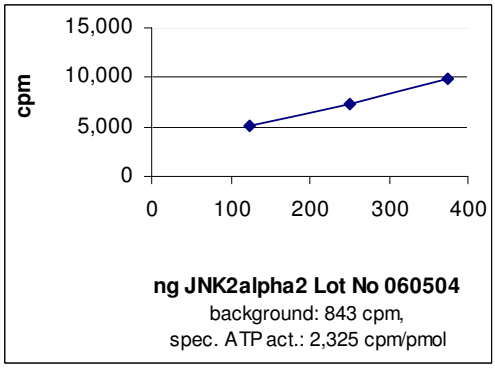
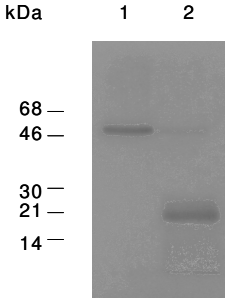
SDS-PAGE analysis



Activity determination



Autophosphorylation



JNK2alpha2 was incubated in the absence (lane 1) or presence of c-jun (1-169, his-tagged) (lane 2) under the assay conditions described below, separated on SDS-PAGE and subjected to autoradiography. In the presence of c-jun as substrate minor autophosphorylation of JNK2alpha2 is detected. > 95 % of phosphate is incorporated into the substrate.

Amino acid sequence information

M13 corresponds to M1 in U09757, note: amino acid 51 of U09757 is D, in L31951 it is S, here it is N (N63)

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MRGSHHHHHH GSMSDSKCDS QFYSVQVADS TFTVLKRYQQ LKPIGSGAQG  50
IVCAAFDTVL GINVAVKKLS RPFQNQTHAK RAYRELVLLK CVNHKNIISL 100
LNVFTPQKTL EEFQDVYLVM ELMDANLCQV IHMELDHERM SYLLYQMLCG 150
IKHLHSAGII HRDLKPSNIV VKSDCTLKIL DFGLARTACT NFMMTPYVVT 200
RYYRAPEVIL GMGYKENVDI WSVGCIMGEL VKGCVIFQGT DHIDQWNKVI 250
EQLGTPSAEF MKKLQPTVRN YVENRPKYPG IKFEELFPDW IFPSESERDK 300
IKTSQARDLL SKMLVIDPDK RISVDEALRH PYITVWYDPA EAEAPPPQIY 350
DAQLEEREHA IEEWKELIYK EVMDWEERSK NGVVKDQPPD AAVSSNATPS 400
QSSSINDISS MSTEQTLASD TDSSLDASTG PLEGCR               436
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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: c-jun (1-169-N-His-fusion protein), 0.1 microgram/ microliter
Protein kinase: JNK2alpha2, 100 ng/microliter in OFAB
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 microCi) [gamma–^{32}P]ATP (3,000 Ci/mmol, e.g. from Hartmann Analytic, Braunschweig, Germany)

Assay procedure

All compounds are pipetted into a microcentrigue tube on ice
1. Add 10 microliter OFAB
2. Add 10 microliter c-jun (1-169-N-His-fusion protein), 0.1 microgram/microliter
3. Add 10 microliter JNK2alpha2 enzyme (1 microgram per assay)
4. Add 10 microliter of the diluted [gamma–^{32}P]ATP
5. Incubate 20 min at 30 °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

alternatively to steps 7. - 12.:
7. Remove 10 microliter and mix with 10 microliter SDS-sample buffer, boil for 3 min
8. Run on 12.5 % SDS PAGE
9. Stain gel with Coomassie Blue, destain, dry for autoradiography. Autoradiograph shows autophosphorylated JNK2alpha2 and phosphorylated c-jun.

References

Kallunki T, Su B, Tsigelny I, Sluss HK, Derijard B, Moore G, Davis R, Karin M 1994. Genes Dev 8: 2996-3007

Su B, Jacinto E, Hibi, M, Karin M, Ben-Neriah Y 1994. Cell 77: 727-736