KinaseDetect ApS
http://www.kinasedetect.com

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

Certificate of Analysis

Product	**JNK2 substrate c-jun N-terminal fragment human recombinant, expressed by an E. coli transcription-translation system, N-His-fusion protein**
Cat No	PKS-001-01
Lot No	130204
Description	Purified human recombinant c-jun N-terminal fragment (amino acids 1 - 169), expressed by an E.coli transcription-translation system *in vitro* with a N-terminal His-tag. c-jun N-terminal fragment serves as substrate for JNK2alpha2. Purified by Ni-agarose affinity chromatography. M.W. 21,077.
Quality	Protein concentration (Bradford with BSA as standard): 0.24 mg/ml Purity: > 95% by SDS PAGE Protease activity (Twining test): none
Form	Liquid. In 25 mM Tris-HCl, 500 mM NaCl, 1 mM DTT, Protease Inhibitor Cocktail Complete (Roche Cat. Nr. 1 697 498, 5 tablets/ l), pH 8.5
Package size	100 microgram
Storage condition	-80 ^{O}C
Shipment conditions	dry ice

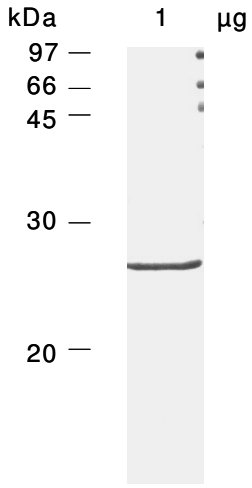
Material for in vitro research use only. Not for pharmaceutical or drug application. Material does not contain any animal products such as albumin.

AVOID FREEZE/THAW CYCLES

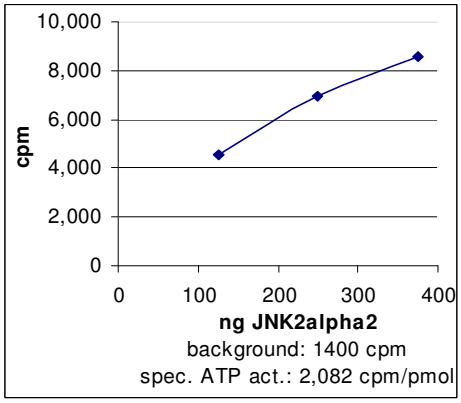
KinaseDetect ApS
http://www.kinasedetect.com

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

SDS-PAGE analysis



Activity determination



1 µg
c-jun(1-169) Lot No 130204 was phosphorylated by increasing amounts JNK2alpha2

Amino acid sequence information

M32 corresponds to M1 in J04111

```
MSGSHHHHHH SSGIEGRGRL IKHMTMASRL EMTAKMETTF YDDALNASFL 50
PSESGPYGYS NPKILKQSMT LNLADPVGSL KPHLRAKNSD LLTSPDVGLL 100
KLASPELERL IIQSSNGHIT TTPTPTQFLC PKNVTDEQEG FAEGFVRALA 150
ELHSQNTLPS VTSAAQPVNG AGMVAPAVAS VAGGSGSGGF SASLHSEPPV 200
```

KinaseDetect ApS
http://www.kinasedetect.com

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

JNK2alpha 2 *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 microg/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 ^{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

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