KinaseDetect ApS
http://www.kinasedetect.com

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

Certificate of Analysis

Product	**ERK1 (MAPK3), active** **human recombinant, expressed in E. coli, N-His-fusion protein**
Cat No	PK-013-01
Lot No	070905
Description	Purified human recombinant ERK1, expressed in E.coli. Active form produced by phosphorylation of purified unactive ERK1 *in vitro* with MEK1. Phosphorylated at Thr202. Suitable for labeling ERK1 substrates. Features a polyhistidine tag to facilitate removal of the enzyme from the reaction mixture. Purifed by Ni-NTA-chromatography. Sequence based calculated M.W. 44,508 Approved HUGO gene symbol: MAPK3 Synonyms: mitogen activated protein kinase 3, extracellular signal regulated kinase 1, ERK1, $p44^{MAPK}$, $p44^{ERK1}$

Quality		
	Protein concentration (Bradford with BSA as standard)	0.5 mg/ml
	Purity	> 95% by SDS PAGE
	Specific activity (* 1 Unit is defined as 1 picomole phosphate transferred to myelin basic protein per min at 30 °C)	257,100 Units*/ mg
	Protease activity (Twining test)	none

Form	Liquid. In 50 mM Tris-HCl, 150 mM NaCl, 1 mM DTT, 50 % glycerol, pH 8.5.
Package size	10 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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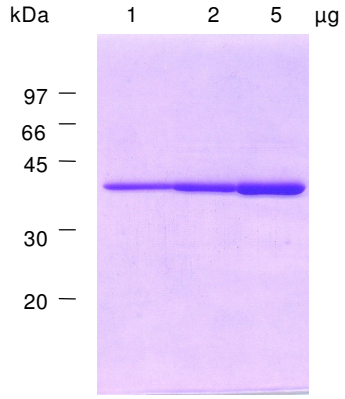
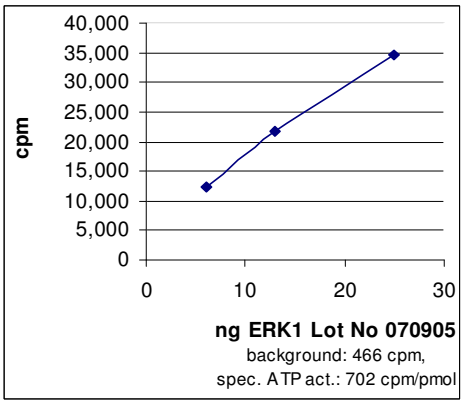
SDS-PAGE analysis



Activity determination



Amino acid sequence information

M13 corresponds to M1 of NM_002746

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MRGSHHHHHH GSMAAAAAQG GGGGEPRRTE GVGPGVPGEV EMVKGQPFDV  50
GPRYTQLQYI GEGAYGMVSS AYDHVRKTRV AIKKISPFEH QTYCQRTLRE 100
IQILLRFRHE NVIGIRDILR ASTLEAMRDV YIVQDLMETD LYKLLKSQQL 150
SNDHICYFLY QILRGLKYIH SANVLHRDLK PSNLLSNTTC DLKICDFGLA 200
RIADPEHDHT GFLTEYVATR WYRAPEIMLN SKGYTKSIDI WSVGCILAEM 250
LSNRPIFPGK HYLDQLNHIL GILGSPSQED LNCIINMKAR NYLQSLPSKT 300
KVAWAKLFPK SDSKALDLLD RMLTFNPNKR ITVEEALAHP YLEQYYDPTD 350
EPVAEEPFTF AMELDDLPKE RLKELIFQET ARFQPGVLEA P          391
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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**:** myelin basic protein, 2 mg/ml
Protein kinase**:** ERK1, 2.5 - 50 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 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 MBP, 2 mg/ ml
3. Add 10 microliter ERK1 (25 - 50 ng/assay)
4. Add 10 microliter of the diluted [gamma–^{32}P]ATP
5. Incubate 10 min at 30 ºC.
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

Reference

Charest DL, Mordret G, Harder KW, Jirik F, Pelech SL 1993 Mol Cell Biol 13: 4679-4690