KinaseDetect ApS
http://www.kinasedetect.com

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

Certificate of Analysis

Product	**ERK2 (MAPK1), active** **human recombinant, expressed in E. coli, N-His-fusion protein**
Cat No	PK-015-01
Lot No	101007
Description	Purified human recombinant ERK2, expressed in E.coli. Active form produced by phosphorylation of the purified unactive ERK1 *in vitro* with MEK1. Phosphorylated at Thr204. Suitable for labeling ERK2 substrates. Features a polyhistidine tag to facilitate removal of the enzyme from the reaction mixture. Sequence based calculated M.W. 44,556 Approved HUGO gene symbol: MAPK1 Synonyms: extracellular signal regulated kinase 2, ERK, ERK2, p41^{mapk}, p42^{MAPK}, p38, MAPK2, PRKM1, PRKM2

Quality		
	Protein concentration (Bradford with BSA as standard)	0.4 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)	470,000 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	5 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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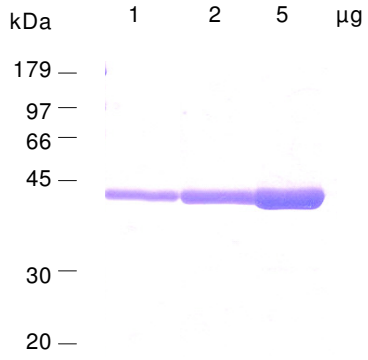
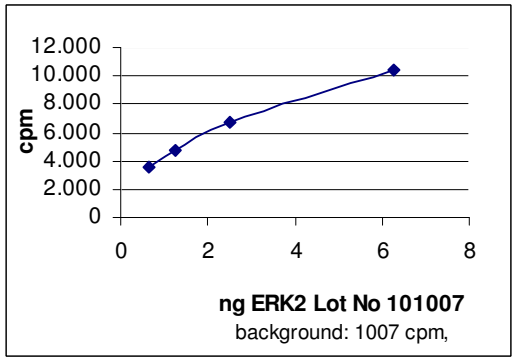
SDS-PAGE analysis



Activity determination



Amino acid sequence information

M30 corresponds to M1 of M84489

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MRGSHHHHHH TDPHASSVPR VDLQGPAANM AAAAAAGAGP EMVRGQVFDV  50
GPRYTNLSYI GEGAYGMVCS AYDNVNKVRV AIKKISPFEH QTYCQRTLRE 100
IKILLRFRHE NIIGINDIIR APTIEQMKDV YIVQDLMETD LYKLLKTQHL 150
SNDHICYFLY QILRGLKYIH SANVLHRDLK PSNLLLNTTC DLKICDFGLA 200
RVADPDHDHT GFLTEYVATR WYRAPEIMLN SKGYTKSIDI WSVGCILAEM 250
LSNRPIFPGK HYLDQLNHIL GILGSPSQED LNCIINLKAR NYLLSLPHKN 300
KVPWNRLFPN ADSKALDLLD KMLTFNPHKR IEVEQALAHP YLEQYYDPSD 350
EPIAEAPFKF DMELDDLPKE KLKELIFEET ARFQPGYRS             388
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In vitro Kinase Assay

Assay Components

Assay-Buffer (AB): 50 mM Tris-HCl, 10 mM NaCl, 10 mM DTT, 0.05 % Brij 35, pH 7.5
Substrate**:** myelin basic protein, 2 mg/ml
Protein kinase**:** ERK2 0.25 - 2.5 ng/ microliter diluted in AB 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 AB
2. Add 10 microliter MBP, 2 mg/ ml
3. Add 10 microliter ERK2 (2.5 - 25 ng/assay)
4. Add 10 microliter of the diluted [gamma–^{32}P]ATP
5. Incubate 10 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