KinaseDetect ApS
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Technical support:
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Certificate of Analysis

Product	**MEK1 (MAP2K1), mutant, active** **human recombinant, expressed in E. coli, N-GST-fusion protein**
Cat No	PK-018-01
Lot No	041007
Description	Purified human recombinant MEK1, expressed in E. coli. Activated by two amino acid exchanges (S218D, S222D). Suitable for labeling MEK1 substrates. Suitable for activation of ERK1 and ERK2. Features a gluthatione-S-transferase (GST) tag to facilitate removal of the enzyme from the reaction mixture. Purified by gluthatione sepharose and gel filtration. Sequence based calculated M.W. 69,730 Approved HUGO gene symbol: MAP2K1 Synonyms: mitogen activated protein kinase kinase 1, MAPKK1

Quality		
	Protein concentration (Bradford with BSA as standard)	0.5 mg/ml
	Purity	> 80% by SDS PAGE
	Specific activity (* 1 Unit is defined as the amount of MEK1 which activates unactive ERK1 (0.3 mg/ml) by 1 U/min using 100 µM ATP at 30 °C. 1 U ERK1 activity is defined as1 pmol phosphate transferred to myelin basic protein (0.2 mg/ml) per min using 125 µM ATP at 30 °C)	543,000Units*/ mg
	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	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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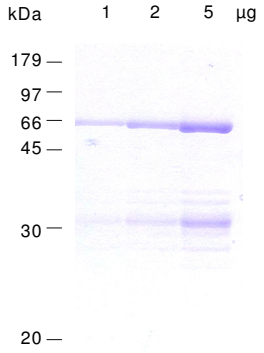
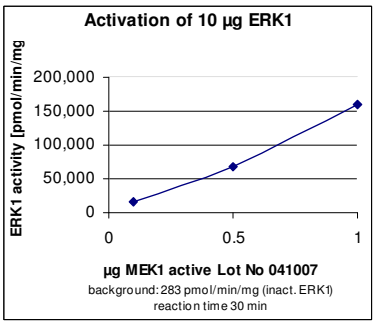
SDS-PAGE analysis



Activity determination



Amino acid sequence information

S227 corresponds to M1 of NM_002755, note: S218 and S222 of NM_002755 are exchanged for D (underlined); the protein contains a thrombin cleavage site between the GST-tag and MEK1 (L221 - S226)

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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 LVPRGSMPKK KPTPIQLNPA PDGSAVNGTS 250
SAETNLEALQ KKLEELELDE QQRKRLEAFL TQKQKVGELK DDDFEKISEL 300
GAGNGGVVFK VSHKPSGLVM ARKLIHLEIK PAIRNQIIRE LQVLHECNSP 350
YIVGFYGAFY SDGEISICME HMDGGSLDQV LKKAGRIPEQ ILGKVSIAVI 400
KGLTYLREKH KIMHRDVKPS NILVNSRGEI KLCDFGVSGQ LIDDMANDFV 450
GTRSYMSPER LQGTHYSVQS DIWSMGLSLV EMAVGRYPIP PPDAKELELM 500
FGCQVEGDAA ETPPRPRTPG RPLSSYGMDS RPPMAIFELL DYIVNEPPPK 550
LPSGVFSLEF QDFVNKCLIK NPAERADLKQ LMVHAFIKRS DAEEVDFAGW 600
LCSTIGLNQP STPTHAAGV                                  619
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Activation of ERK1

Note: the same procedure can be applied for activation of ERK2**.** The volume of the reaction depends on the amount of ERK1/ERK2 to be activated. Final concentration of ERK1/ERK2 should be 0.3 mg/ml.

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

ERK1 dilution buffer: 50 mM Tris-HCl, 150 mM NaCl, 2 mM DTT, pH 8.0

Protein kinase MEK1

Protein kinase ERK1, unactive in 50 mM Tris-HCl, 150 mM NaCl, 2 mM DTT, 5 mM EDTA pH 8.0

Magnesium/ATP Cocktail**:** 75 mM $MgCl_2$, 500 microM ATP

Assay Procedure

All compounds are pipetted into a microcentrigue tube on ice

1. Add 1/5 volume OFAB
2. Add ERK1 (final concentration 0.3 mg/ml)
3. Add MEK1 (1/10th the amount of ERK1)
4. Fill up with ERK1 dilution buffer to 4/5 volume
5. Add 1/5 volume of Magnesium/ATP Cocktail
6. Incubate 30 min at 30 ºC.
7. Stop the reaction by setting samples on ice

The reaction might be dialyzed against 100 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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ERK1 *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 - 10 mg/ml diluted in OFAB directly before use

Magnesium/ATP Cocktail**:** 75 mM $MgCl_2$, 500 microM ATP

Diluted [gamma–^{32}P]ATP: Mix 197 microl 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 - 100 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

References

Alessandrini A., Greulich H, Huang W, Erikson RL (1996) J Biol Chem 271:31612-31618

Schramek H, Feifel E, Healy E, Pollak V (1997) J Bio Chem 272: 11426-11433

Allen LF, Seboldt-Leopold J, Meyer MB (2003) Sem Oncol 30, Suppl 16: 105-116