

#7416 Store at -80°C

ERK1 Kinase

✓ 5 µg

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This product is for *in vitro* research use only and is not intended for use in humans or animals.

Description: Purified recombinant full-length human ERK1 (Met1-Pro379) kinase, supplied as a tag-free protein.

Background: Mitogen-activated protein kinases (MAPKs) are a widely conserved family of serine/threonine protein kinases involved in many cellular programs such as cell proliferation, differentiation, motility, and death. The p44/42 MAPK (ERK1/2) signaling pathway can be activated in response to a diverse range of extracellular stimuli including mitogens, growth factors, and cytokines (1-3) and is an important target in the diagnosis and treatment of cancer (4). Upon stimulation, a sequential three-part protein kinase cascade is initiated, consisting of a MAP kinase kinase kinase (MAP3KK), a MAP kinase kinase (MAP2KK), and a MAP kinase. While multiple ERK1/2 MAP3Ks have been identified, including the Raf family, Mos, and Tpl2/Cot, MEK1 and MEK2 are the primary MAP2Ks in this pathway (5,6). MEK1 and MEK2 activate ERK1/p44 and ERK2/p42 through phosphorylation of activation loop residues Thr202/Tyr204 and Thr185/Tyr187, respectively. Several downstream targets of ERK1/2 have been identified, including p90RSK (7) and the transcription factor Elk-1 (8,9). ERK1/2 are negatively regulated by a family of dual-specificity (Thr/Tyr) MAPK phosphatases, known as DUSPs or MKPs (10), along with MEK inhibitors such as U0126 and PD98059.

Source/Purification: The kinase was produced using a baculovirus expression system with a construct expressing full-length human ERK1 (Met1-Pro379) (GenBank Accession No. NM_002746). The protein was activated by active MEK1 *in vitro*. The protein was purified after being activated.

Quality Control: The theoretical molecular weight of the ERK1 protein is 44 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. ERK1 kinase activity was determined using a radiometric assay [Fig.2].

Background References:

- (1) Roux, P.P. and Blenis, J. (2004) *Microbiol Mol Biol Rev* 68, 320–44.
- (2) Baccarini, M. (2005) *FEBS Lett* 579, 3271–7.
- (3) Meloche, S. and Pouyssegur, J. (2007) *Oncogene* 26, 3227–39.
- (4) Roberts, P.J. and Der, C.J. (2007) *Oncogene* 26, 3291–310.
- (5) Rubinfeld, H. and Seger, R. (2005) *Mol Biotechnol* 31, 151–74.
- (6) Murphy, L.O. and Blenis, J. (2006) *Trends Biochem Sci* 31, 268–75.
- (7) Dalby, K.N. et al. (1998) *J Biol Chem* 273, 1496–505.
- (8) Marais, R. et al. (1993) *Cell* 73, 381–93.
- (9) Kortenjann, M. et al. (1994) *Mol Cell Biol* 14, 4815–24.
- (10) Owens, D.M. and Keyse, S.M. (2007) *Oncogene* 26, 3203–13.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione. Store at -80°C.

Keep on ice during use.
Avoid repeated freeze-thaw cycles.

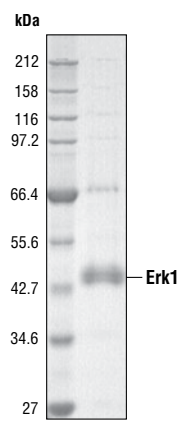


Figure 1. The purity of the ERK1 protein was analyzed using SDS/PAGE followed by Coomassie stain.

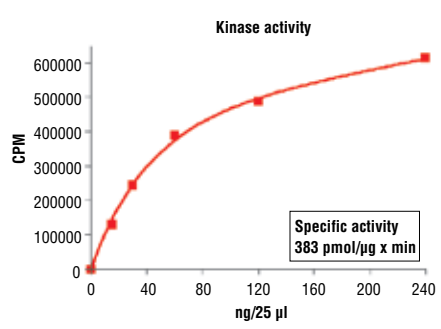


Figure 2. ERK1 kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM MgCl₂, 0.05 mM DTT, 50 µM ATP, Substrate: MBP 200 ng/µL and recombinant ERK1: variable.

Protocol for ERK1 Kinase Assay

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

- 1. Kinase Buffer (5X)**
25 mM MOPS, pH 7.2
12.5 mM β -glycerophosphate
5 mM EGTA
2 mM EDTA
25 mM $MgCl_2$
0.25 mM DTT
- 2.** ATP (10 mM) #9804
- 3.** ^{32}P - γ ATP
- 4.** MBP (1.0 μ g/ μ l)

B Suggested Protocol

- 1.** Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 μ M ATP.
- 2.** Dilute [^{32}P] ATP to 0.16 μ Ci/ μ l [^{32}P] ATP with 250 μ M ATP solution.
- 3.** Transfer enzyme from $-80^\circ C$ to ice. Allow enzyme to thaw on ice.
- 4.** Dilute ERK1 protein (100 ng/ μ l concentration) to 20 ng/ μ l with 1X assay buffer followed by 2-fold serial dilutions.
- 5.** To start the reaction combine 10 μ l diluted ERK1 kinase solution, 10 μ l MBP (1.0 μ g/ μ l) and 5 μ l 0.16 μ Ci/ μ l [^{32}P] ATP solution.

Final Assay Conditions

- 5 mM MOPS, pH 7.2
 - 2.5 mM β -glycerophosphate
 - 1 mM EGTA
 - 5 mM $MgCl_2$
 - 0.05 mM DTT
 - 400 ng/ μ l MBP
- 6.** After 15 minutes terminate reaction by spotting 20 μ l of the reaction mixture onto phosphocellulose P81 paper.
 - 7.** Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
 - 8.** Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
 - 9.** Count samples in a scintillation counter.

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