Store at –80°C

#7591

Erk2 Kinase

☑5 µg

Cell Signaling TECHNOLOGY®

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New 11/07

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 Erk2 (Met1_Ser360) kinase, supplied as a GST fusion 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 (MAPKKK), a MAP kinase kinase (MAPKK), 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 MAPKKs 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 *E. coli* cells with a construct expressing full-length human Erk2 (Met1-Ser360) (GenBank Accession No. NM_002745) with an amino-terminal GST tag. The protein was activated by active MEK1 *in vitro* and purified after activation.

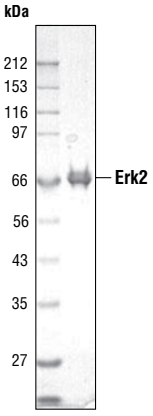

Figure 1. The purity of the Erk2 protein was analyzed using SDS/PAGE followed by Coomassie stain.

Quality Control: The theoretical molecular weight of the Erk2 protein is 68 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. Erk2 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 Pouysségur, 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.

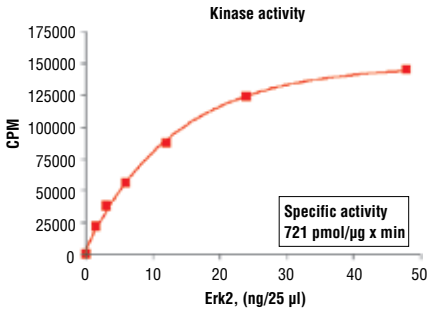

Figure 2. Erk2 kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM $MgCl_2$, 0.05 mM DTT, 50 µM ATP, Substrate: MBP 200 ng/µL, and Recombinant Erk2: variable.

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.

Companion Products:
Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Serine/Threonine Kinase Substrate Screening Kit #7400

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Protocol for Erk2 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-γATP
4. MBP (0.5 µg/µl)

B Suggested Protocol

1. Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 µM ATP.
2. Dilute [^{32}p] ATP to 0.16 µCi/µl [^{32}p] ATP with 250 µM ATP solution.
3. Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
4. Dilute Erk2 kinase protein (100 ng/µl concentration) to 20 ng/µl with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 µl diluted Erk2 kinase solution, 10 µl MBP (0.5 µg/µl), and 5 µl 0.16 µCi/µl [^{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
200 ng/µl MBP

6. After 15 minutes terminate reaction by spotting 20 µl 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.

Cell Signaling Technology offers a full line of protein kinases, substrates, and antibody detection reagents for high throughput screening. Please direct all inquiries to: drugdiscovery@cellsignal.com.

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