ERK2, Active

Full-length recombinant protein expressed in E. coli cells

Catalog # M28-10G
Lot # M048-2

Product Description

Recombinant full-length human ERK2 was expressed by E.coli cells using an N-terminal GST tag and activated by MEK1 in vitro. The gene accession number is NM_002745.

Gene Aliases

MAP1, P42MAPK, MAPK2, PRKM2, PRKM1, p41mapk, ERT1, p40, p41, ERK

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Scientific Background

ERK2 is a protein serine/threonine kinase that is a member of the extracellular signal-regulated kinases (ERKs) which are activated in response to numerous growth factors and cytokines (1). Activation of ERK2 requires both tyrosine and threonine phosphorylation that is mediated by MEK. ERK2 is ubiquitously distributed in tissues with the highest expression in heart, brain and spinal cord. Activated ERK2 translocates into the nucleus where it phosphorylates various transcription factors (e.g., Elk-1, c-Myc, c-Jun, c-Fos, and C/EBP beta).

References


ERK2, Active

Full-length recombinant protein expressed in E. coli cells

Specific Activity

The specific activity of ERK2 was determined to be 682 nmol/min/mg as per activity assay protocol.

Purity

The purity of ERK2 was determined to be >95% by densitometry, approx. MW 68kDa.

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com

FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.
Activity Assay Protocol

Reaction Components

**Active Kinase (Catalog #: M28-10G)**
Active ERK2 (0.1mg/µl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active ERK2 for optimal results).

**Kinase Dilution Buffer III (Catalog #: K23-09)**
Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 30ng/µl BSA solution.

**Kinase Assay Buffer I (Catalog #: K01-09)**
Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

**[^3P]-ATP Assay Cocktail**
Prepare 250µM [^3P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [^3P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at –20°C.

**10mM ATP Stock Solution (Catalog #: A50-09)**
Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200µl aliquots at –20°C.

**Substrate (Catalog #: M42-51N)**
Myelin basic protein (MBP) diluted in distilled H₂O to a final concentration of 1mg/ml.

**Assay Protocol**

1. Thaw [^3P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
2. Thaw the Active ERK2, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
   - Component 1. 10µl of diluted Active ERK2 (Catalog #M28-10G)
   - Component 2. 5µl of 1mg/ml stock solution of substrate (Catalog #M42-51N)
   - Component 3. 5µl distilled H₂O (4°C)
4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
5. Initiate the reaction by the addition of 5 µl [^3P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
6. After the 15 minute incubation period, terminate the reaction by spotting 20 µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

**Calculation of [^3P]-ATP Specific Activity (SA) (cpm/pmol)**

Specific activity (SA) = cpm for 5 µl [^3P]-ATP / pmoles of ATP (in 5 µl of a 250 µM ATP stock solution, i.e., 1250 pmoles)

**Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)**

Corrected cpm from reaction / [(SA of ^3P-ATP in cpm/pmol)* (Reaction time in min)* (Enzyme amount in µg or mg)] / [Reaction Volume / (Spot Volume)]

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com

FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.