Recombinant Human Active ERK1 Protein, CF

Catalog Number: 1879-KS-010
Lot Number: 1757168

SPECIFICATIONS AND USE

Source	Recombinant full-length, tag-free human ERK1 was expressed in *E.coli* cells and activated by active MEK1 *in vitro*. The gene accession number is NM_002746.
Molecular Mass	The approximate molecular weight is 44 kDa.
Purity	The purity was determined to be > 80% by densitometry.
Formulation	Supplied in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 0.1 mM PMSF and 25% Glycerol.
Size	10 µg
Concentration	0.1 µg/µL
Activity	The specific activity of ERK1 was determined to be 42 nmol /min/mg as per Activity Assay Protocol and was equivalent to 382 nmol/min/mg as per radiometric assay.
Storage	This product is stable at ≤ -70 °C for up to one year from the date of receipt. For optimal storage, aliquot into smaller quantities after centrifugation and store at recommended temperature. **Avoid repeated freeze-thaw cycles.**

BACKGROUND

ERK1 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 ERK1 requires both tyrosine and threonine phosphorylation that is mediated by MEK. ERK1 is ubiquitously distributed in tissues with the highest expression in heart, brain and spinal cord. Activated ERK1 translocates into the nucleus where it phosphorylates various transcription factors (e.g., Elk1, c-Myc, c-Jun, c-Fos, and C/EBPβ).

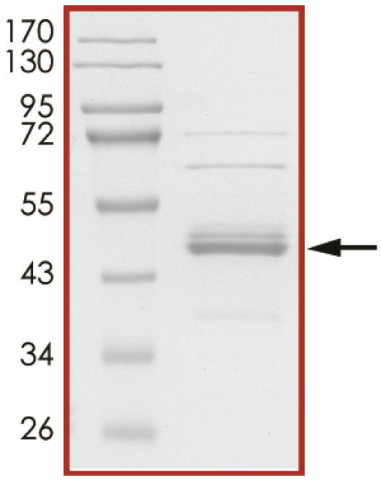

Figure 1: The purity of ERK1 was determined to be > 80% by densitometry. Calculated molecular weights is approximately 44 kDa. Observed molecular weights is approximately 48 kDa.

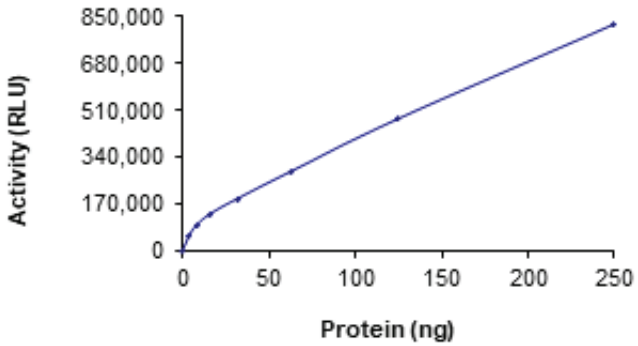

Figure 2: The specific activity of ERK1 was determined to be 42 nmol /min/mg as per activity assay protocol and was equivalent to 382 nmol/min/mg as per radiometric assay.

REFERENCES

1. Boulton, T.G. *et al.* (1991) Biochemistry **30**:278.

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ACTIVITY ASSAY PROTOCOL

Solutions Required

Active Kinase - Active ERK1 (0.1 µg/µL) diluted with Kinase Dilution Buffer IX and assayed as outlined in Figure 2.
Note: *These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.*

Kinase Assay Buffer III (5X) - 200 mM Tris-HCl, pH 7.4, 100 mM $MgCl_2$, and 0.5 mg/mL BSA. Add fresh DTT prior to use to a final concentration of 250 µM.

Kinase Dilution Buffer IX (1X) - Kinase Assay Buffer III diluted at a 1:4 ratio (5X dilution) with cold distilled water. Add fresh DTT prior to use to a final concentration of 50 uM.

ADP-Glo™ Kinase Assay Kit - 10 mM ATP Solution, 10 mM ADP Solution, ADP-Glo™ Reagent, Kinase Detection Reagent.

Substrate - Myelin Basic Protein (MBP) diluted in 100 mM MOPS buffer (pH 6.5) to a final concentration of 0.5 mg/mL.

ASSAY PROCEDURE

The ERK1 assay is performed using the ADP-Glo Kinase Assay Kit which quantifies the amount of ADP produced by the ERK1 reaction. The ADP-Glo Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

1. Thaw the Active ERK1, Kinase Assay Buffer III (5X), and Substrate on ice. Prepare a 15 µL enzyme dilution using Kinase Dilution Buffer IX (1X), at the desired concentration, in a pre-chilled 96-well plate.
2. Prepare a substrate/ATP mixture as follows (25 µM ATP example):

Reaction Component	Amount
10 mM ATP Solution	1 µL
Kinase Assay Buffer III (5X)	79 µL
Substrate at 0.5 mg/mL	80 µL

3. Transfer the following reaction components prepared in Steps 1 and 2 to a 384-well opaque plate bringing the reaction volume up to 5 µL:

Component 1	3 µL of diluted Active ERK1.
Component 2	2 µL of Substrate/ATP mix as prepared in the table above. This initiates the reaction.

4. Set up the blank control as outlined in step 2, excluding the addition of the kinase. Replace the kinase with an equal volume of Kinase Dilution Buffer IX (1X).
5. Incubate at ambient temperature for 40 minutes.
6. After the 40-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 5 µL of ADP-Glo Reagent. Spin down and shake the 384-well plate. Then incubate the reaction mixture for another 40 minutes at ambient temperature.
7. Add 10 µL of the Kinase Detection Reagent to the 384-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.
8. Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader.
9. Determine the corrected activity (RLU) by removing the blank control value (step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of Specific Activity of ADP (RLU/pmol)

From ATP-ADP conversion curve, determine RLU/pmol of ADP

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

Corrected RLU from reaction / [(SA of ADP in RLU/pmol) * (Reaction time in min) * (Enzyme amount in µg or mg)]