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Catalog #	Aliquot Size
M31-10G-05	5 µg
M31-10G-10	10 µg

ERK3 (MAPK6), Active

Human recombinant protein expressed in Sf9 cells

Catalog # M31-10G
Lot # C2078-9

Product Description

Recombinant full-length human ERK3 (MAPK6) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. This gene accession number is NM_002748.

Gene Aliases

ERK3; MAPK6; HsT17250; p97MAPK; PRKM6

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 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.

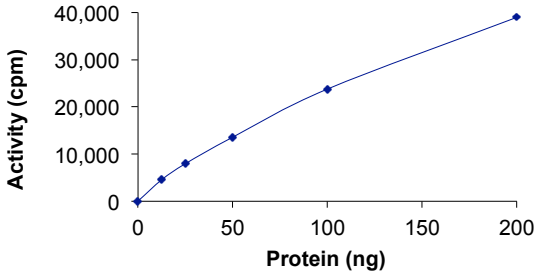
Scientific Background

ERK3 (MAPK6) or mitogen-activated protein kinase 6 is a member of the Ser/Thr protein kinase family, and is most closely related to mitogen-activated protein kinases (MAP kinases) which also known as extracellular signal-regulated kinases (ERKs) that are activated through protein phosphorylation cascades and act as integration points for multiple biochemical signals and tyrosine phosphorylated in response to insulin and NGF (1). ERK3 is highly expressed in various human tissues, most abundantly in skeletal muscle (2).

References

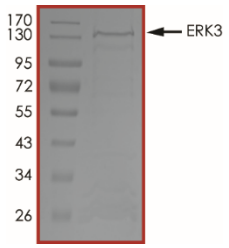
1. Boulton, T. G.et.al: ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. Cell 65: 663-675, 1991.
2. Meloche, S. et.al: Primary structure, expression and chromosomal locus of a human homolog of rat ERK3. Oncogene 13: 1575-1579, 1996.

Specific Activity



The specific activity of ERK3 was determined to be **22 nmol /min/mg** as per activity assay protocol.

Purity



The purity of ERK3 protein was determined to be **>75%** by densitometry, approx. MW **~135kDa**.

ERK3 (MAPK6), Active

Human recombinant protein expressed in Sf9 cells

Catalog #	M31-10G
Specific Activity	22 nmol/min/mg
Lot #	C2078-9
Purity	>75%
Concentration	0.05 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	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. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: M31-10G)

Active ERK3 (MAPK6) (0.05μg/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 ERK3 (MAPK6) 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 50ng/μl BSA solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250μM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [^{33P}]-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

Unactive MAPKAPK5 (Catalog #: M42-14G) was activated using ERK3 (Catalog #: M31-10G) and then HSP27tide peptide (Catalog #: H31-58) was added as the activated MAPKAPK5's substrate.

Assay Protocol

Step 1. Thaw the Active ERK3 Kinase Assay Buffer and Unactive MAPKAPK5 on ice. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20μl:

- **Component 1.** 7μl of diluted Active ERK3 (Catalog #M31-10G)
- **Component 2.** 8μl of Unactive MAPKAPK5 (0.1μg/μl) (Catalog #M42-14G)
- **Component 3.** 5μl of Kinase Assay Buffer (Catalog #K01-09)

Step 2. Start the reaction by the addition of 5 μl ATP (250μM) and incubate in a water bath at 30°C for 25 minutes.

Step 3. After the 25 minute incubation period, remove 5μl and add to the following reaction components bringing the initial reaction volume up to 20μl:

- **Component 1.** 5μl of reaction mixture
- **Component 2.** 2.5μl of HSP27tide peptide (Catalog #H31-58)
- **Component 3.** 12.5μl distilled H_2O (4°C)

Step 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 H_2O.

Step 5. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area. Initiate the reaction by the addition of 5 μl [^{33}P]-ATP Assay Cocktail bringing the final volume up to 25μl and incubate the mixture in a water bath at 30°C for 15 minutes.

Step 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.

Step 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 H_2O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

Step 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 [P^{33}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μl [^{33}P]-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 ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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