

LIMK1, Active

Recombinant human protein expressed in Sf9 cells

Catalog # L04-11G

Lot # O988-3

Product Description

Recombinant human LIMK1 (285-638) was expressed by baculovirus in Sf9 cells using an N-terminal GST tag. The gene accession number is [NM_002314](#).

Gene Aliases

LIMK, KIZ

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.

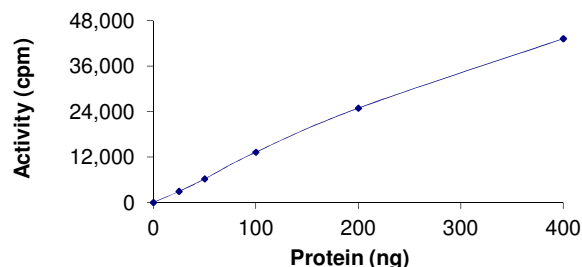
Scientific Background

LIMK1 or LIM domain kinase 1 contains a unique combination of 2 N-terminal LIM domain and a C-terminal protein kinase domain. LIM domains are highly conserved cysteine-rich structures containing 2 zinc fingers that can bind to DNA/RNA as well as mediating protein-protein interactions (1). LIMK1 is thought to be a component of an intracellular signaling pathway that may be involved in brain development especially development of nerve cells. LIMK1 may play an important role in areas of the brain that are responsible for processing visual-spatial information (visuospatial constructive cognition). LIMK1 can regulate aspects of the cytoskeleton, the structural framework that helps to determine cell shape, size, and movement (2).

References

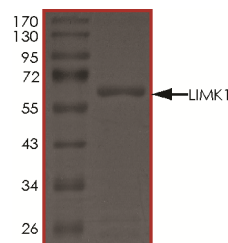
1. Davila, M. et al: LIM kinase 1 is essential for the invasive growth of prostate epithelial cells: implications in prostate cancer. J Biol Chem. 2003;19;278(38):36868-75.
2. Davila, M. et al: Expression of LIM kinase 1 is associated with reversible G1/S phase arrest, chromosomal instability and prostate cancer. Mol Cancer. 2007;8;6:40.

Specific Activity



The specific activity of LIMK1 was determined to be **8 nmol/min/mg** as per activity assay protocol.

Purity



The purity of LIMK1 was determined to be **>95%** by densitometry. Approx. MW **65kDa**.

LIMK1, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	L04-11G
Specific Activity	8 nmol/min/mg
Specific Lot Number	O988-3
Purity	>95%
Concentration	0.1 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 #: L04-11G)

Active LIMK1 (0.1 µg/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 LIMK1 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) 50ng/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.

[³³P]-ATP Assay Cocktail

Prepare 250µM [³³P]-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 (Catalog #: M42-51N)

MBP protein substrate diluted in distilled H₂O to a final concentration of 1mg/ml.

Note: Cofilin 1 Protein (Catalog #C37-54H) can be also used as a substrate for this target and it shows good activity.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active LIMK1, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 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 LIMK1 (Catalog #L04-11G)
 - Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #M42-51N)
 - Component 3.** 5µl distilled H₂O (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 H₂O.
- Step 5.** Initiate the reaction by the addition of 5µl [³³P]-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 20µl 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 H₂O) 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 [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [³³P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1250 pmoles)

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

Corrected cpm from reaction / [(SA of ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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