

ALK1, Active

Recombinant human protein expressed in Sf9 cells

Catalog # A09-11G

Lot # P1844-9

Product Description

Recombinant human ALK1 (144-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag and further purified by chromatograph columns. The gene accession number is [NM_000020](#).

Gene Aliases

ACVRL1, ACVRLK1, ALK1, HHT, HHT2, ORW2, SKR3, ALK1, TSR-1

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

ALK1 is a serine/threonine-protein kinase receptor R3 precursor that mediates signal by TGF β superfamily (1). ALK1 functions as a TGF β type I receptor in endothelial cells and is responsible for human hereditary hemorrhagic telangiectasia (HHT) type II. Distinct Smad proteins (i.e., Smad2/Smad3 and Smad1/Smad5) show interaction with ALK1 and mediate TGF β signaling. Northern blot and RT-PCR analysis show that ALK1 specifically induces expression of Smad6, Smad7, Id1, Id2, endoglin, STAT1, and interleukin 1 receptor in endothelial cells. ALK1 expression in inflammatory myofibroblastic tumor of the urinary bladder have also has been reported (2).

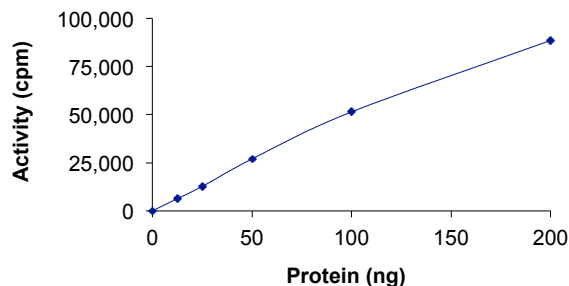
References

- Ota, T. et al: Targets of transcriptional regulation by two distinct type I receptors for transforming growth factor-beta in human umbilical vein endothelial cells. *J. Cell Physiol.* 2002;193(3):299-318.
- Tsuzuki, T. et al: ALK1 expression in inflammatory myofibroblastic tumor of the urinary bladder. *Am. J. Surg. Pathol.* 2004; 28(12):1609-14.

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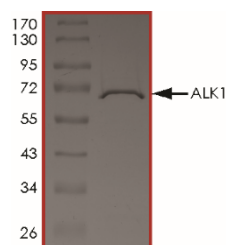
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Specific Activity



The specific activity of ALK1 was determined to be **25 nmol /min/mg** as per activity assay protocol.

Purity



The purity of ALK1 was determined to be **>95%** by densitometry, approx. MW **64kDa**.

ALK1, Active

Recombinant protein expressed in Sf9 cells

Catalog #	A09-11G
Specific Activity	25 nmol/min/mg
Specific Lot #	P1844-9
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.

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: A09-11G)

Active ALK1 (0.1µg/µl) diluted with Kinase Dilution Buffer IV (Catalog #: K24-09) 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 ALK1 for optimal results).

Kinase Dilution Buffer IV (Catalog #: K24-09)

Kinase Assay Buffer II (Catalog #: K02-09) diluted at a 1:4 ratio (5X dilution) with final 50ng/µl BSA solution.

Kinase Assay Buffer II (Catalog #: K02-09)

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 20mM MgCl₂, 12.5mM MnCl₂, 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 II (Catalog #: K02-09). 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 II (Catalog #: K02-09). Store 200µl aliquots at -20°C.

Substrate (Catalog #: C03-54BN)

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

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active ALK1, 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 ALK1 (Catalog #A09-11G)
 - Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #C03-54BN)
 - 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 5 µl [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 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 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 5 µl [³³P]-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 ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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