

ALK (F1174L), Active

Human recombinant protein expressed in Sf9 cells

Catalog # **A19-12EG**

Lot # Y1068-1

Product Description

Recombinant human ALK (F1174L) (1060-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_004304](#).

Gene Aliases

ALK (Ki-1), CD246, NBLST3, TFG/ALK

Formulation

Recombinant protein stored in 50mM sodium phosphate, pH 7.0, 300mM NaCl, 150mM imidazole, 0.1mM PMSF, 0.25mM DTT, 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

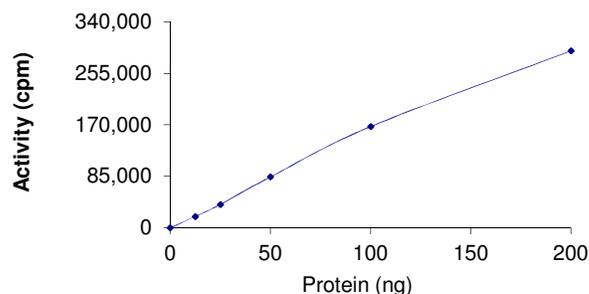
ALK or anaplastic lymphoma kinase is a receptor tyrosine kinase that was originally identified as a member of the insulin receptor subfamily that acquires transforming capability when truncated and fused to NPM (nucleophosmin) in the t(2;5) chromosomal rearrangement associated with ALCL. Many chromosomal rearrangements leading to enhanced ALK activity have been described and are implicated in a number of cancer types (1). In the nervous system, ALK in the presence of ligand appears essential for axonal guidance, whereas in the absence of ligand, ALK expression can lead to developmental neuronal apoptosis (2).

References

- Palmer R H, et al: Anaplastic lymphoma kinase: signalling in development and disease. *Biochem J.* 2009 May 27;420(3):345-61.
- Allouche M: ALK is a novel dependence receptor: potential implications in development and cancer. *Cell Cycle.* 2007 Jul 1;6(13):1533-8.

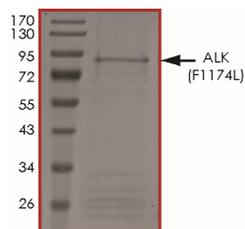
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Specific Activity



The specific activity of ALK (F1174L) was determined to be **112 nmol/min/mg** as per activity assay protocol.

Purity



The purity of ALK (F1174L) was determined to be **>75%** by densitometry, approx. MW **90kDa**.

ALK (F1174L), Active

Human recombinant protein expressed in Sf9 cells

Catalog # A19-12EG
Specific Activity 112 nmol/min/mg
Lot # Y1068-1
Purity >75%
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 #: A19-12EG)

Active ALK (F1174L) (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 ALK (F1174L) 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 I (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 I (Catalog #: K01-09). Store 200µl aliquots at -20°C.

Substrate (Catalog #: I15-58)

IGF1Rtide synthetic peptide substrate (KKKSPGEYVNIEFG) 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 ALK (F1174L), 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 ALK (F1174L) (Catalog # A19-12EG)
 - Component 2.** 5µl of 1 mg/ml stock solution of substrate (Catalog #I15-58)
 - 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 (cpm) 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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