

TFG-ALK (Tex4Aex20), Active

Recombinant human protein expressed in Sf9 cells

Catalog # A19-19KG

Lot # M2720-6

Product Description

Recombinant human fusion protein TFG (1-138a.a., exons1-4)-ALK (1058a.a.-end, exons20-29) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number of TFG is [NM_001195478](#) and ALK is [NM_004304](#).

Gene Aliases

TFG: HMSNP; SPG57; TF6

ALK: ALK (Ki-1), CD246

Formulation

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

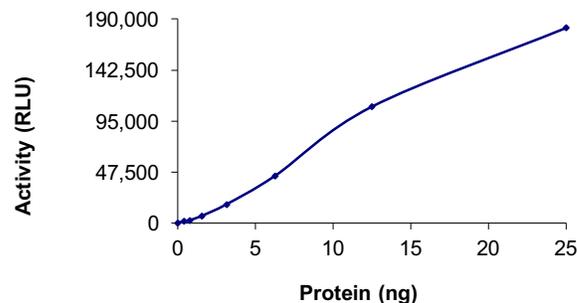
Anaplastic large cell lymphomas are associated with chromosomal aberrations involving the anaplastic lymphoma kinase (ALK) gene, resulting in the expression of novel chimeric ALK proteins with transforming properties. In most of these tumors, the t(2;5)(p23;q35) generates the NPM-ALK fusion gene. Genes other than NPM have been demonstrated to fuse to the ALK gene. Two different ALK rearrangements involving the TRK-fused gene (TFG) in which the same portion of ALK was fused to different length fragments of the 5' TFG region. These two rearrangements encoded chimeric proteins of 85 kd (TFG-ALK(S)) and 97 kd (TFG-ALK(L)), respectively. TFG can provide an alternative to NPM as a fusion partner responsible for activation of the ALK and the pathogenesis of ALCL.

References

- Hernández L. et al: TRK-fused gene (TFG) is a new partner of ALK in anaplastic large cell lymphoma producing two structurally different TFG-ALK translocations. *Blood*. 1999 Nov 1;94(9):3265-8.
- Hernández L. et al: Diversity of genomic breakpoints in TFG-ALK translocations in anaplastic large cell lymphomas: identification of a new TFG-ALK(XL) chimeric gene with transforming activity. *Am J Pathol*. 2002 160(4):1487-94. 2002.

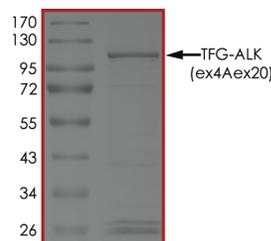
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Specific Activity



The specific activity of TFG-ALK (Tex4Aex20) was determined to be **22 nmol/min/mg** as per activity assay protocol.

Purity



The purity of TFG-ALK (Tex4Aex20) protein was determined to be **>85%** by densitometry, approx. MW **106 kDa**.

TFG-ALK (Tex4Aex20), Active

Recombinant human protein expressed in Sf9 cells

Catalog # A19-19KG

Specific Activity 22 nmol/min/mg

Lot # M2720-6

Purity >85%

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.

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: A19-19KG)

Active TFG-ALK (Tex4Aex20) (0.05µg/µl) diluted with Kinase Dilution Buffer X (1x) (Catalog #: K20-09) 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 TFG-ALK for optimal results).

Kinase Assay Buffer III (5x) (Catalog #: K03-09)

Buffer components: 200mM Tris-HCl, pH 7.4, 100mM MgCl₂ and 0.5mg/ml BSA. Add fresh DTT prior to use to a final concentration of 250µM.

Kinase Dilution Buffer X (1x) (Catalog #: K20-09)

Kinase Assay Buffer III (Catalog #: K03-09) with 12.5mM MnCl₂ diluted at a 1:4 ratio (5X dilution) with cold water. Add fresh DTT to the aliquot prior to use to a final concentration of 50µM.

ADP-Glo™ Kinase Assay Kit (Promega, Cat # V9101)

ATP solution, 10 mM
ADP solution, 10 mM
ADP-Glo™ Reagent
Kinase Detection Reagent

Substrate (Catalog #: I15-58)

IGF1Rtide synthetic peptide substrate (KKKSPGEYVNIIEFG) diluted in distilled H₂O to a final concentration of 0.2 mg/ml.

Cofactor: 2.5M MnCl₂ (Catalog #: M40-09-25)

Diluted to a working concentration of 0.1M in distilled H₂O.

Assay Protocol

The TFG-ALK assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the TFG-ALK 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.

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

Component	Amount (µL)	Component	Amount (µL)
10µM ATP Solution	1.25	Substrate at 1mg/mL	50
Kinase Assay Buffer III (5x)	46.75	0.1M MnCl ₂	2

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

Component 1.	3µl of diluted Active TFG-ALK (Catalog # A19-19KG).
Component 2.	2µl of Substrate/ATP mix as prepared in the table above. This initiates the reaction.

- Step 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 X (1x).
- Step 5.** Incubate at ambient temperature for 40 minutes.
- Step 6.** After the 40-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 5µl of ADP-Glo™ Reagent. Spin down and shake the 384-well plate. Then incubate the reaction mixture for another 40 minutes at ambient temperature.
- Step 7.** Then add 10µl of the Kinase Detection Reagent to the 384-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.
- Step 8.** Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader (Promega; Cat# E7061).
- Step 9.** Determine the corrected activity (RLU) by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of Specific Activity of ADP (RLU/pmol)

From ADP standard 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)

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