

TFG-ALK (Tex5Aex20), Active

Recombinant human protein expressed in Sf9 cells

Catalog # A19-19CG

Lot # K1779-2

Product Description

Recombinant human fusion TFG (1-193a.a., exons1-5)-ALK (1058a.a.-end, exons20-29) protein 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

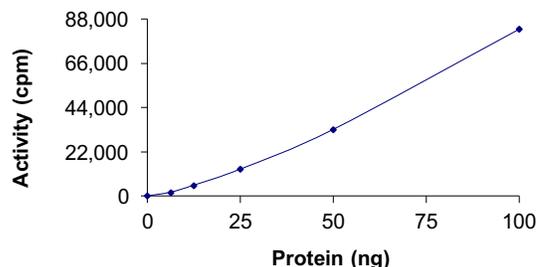
Anaplastic large cell lymphomas are associated with chromosomal aberrations involving the anaplastic lymphoma kinase (ALK) gene, resulting in the expression of novel chimeric ALK proteins with transforming properties. In most of these tumors, the t(2;5)(p23;q35) generates the NPM-ALK fusion gene. Genes 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.

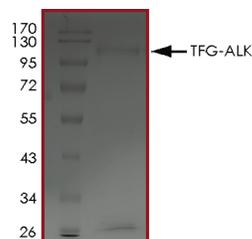
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Specific Activity



The specific activity of TFG-ALK (Tex5Aex20) was determined to be **28 nmol/min/mg** in a coupled assay as per activity assay protocol.

Purity



The purity of TFG-ALK (Tex5Aex20) protein was determined to be **>70%** by densitometry, approx. MW **115 kDa**.

TFG-ALK (Tex5Aex20), Active

Recombinant human protein expressed in Sf9 cells

Catalog #	A19-19CG
Specific Activity	28 nmol/min/mg
Lot #	K1779-2
Purity	>70%
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 #: A19-19CG)

Active TFG-ALK (Tex5Aex20) (0.05µg/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 TFG-ALK (Tex5Aex20) 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 #: I15-58)

IGF1Rtide synthetic peptide substrate (KKKSPGEYVNIIEFG) 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 TFG-ALK (Tex5Aex20), 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 TFG-ALK (Tex5Aex20) (Catalog # A19-19CG)
 - 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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