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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
A19-19LG -05	5 µg
A19-19LG -10	10 µg

TPM1-ALK, Active

Human recombinant protein expressed in Sf9 cells

Catalog # A19-19LG
Lot # M2982-7

Product Description

Recombinant human fusion protein TPM1 (1-257)-ALK (1058-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The TPM1 gene accession number is NM_001018005 and ALK is NM_004304.

Gene Aliases

TPM1: C15orf13; CMD1Y; CMH3; HEL-S-265; HTM-alpha; LVNC9; TMSA;
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.

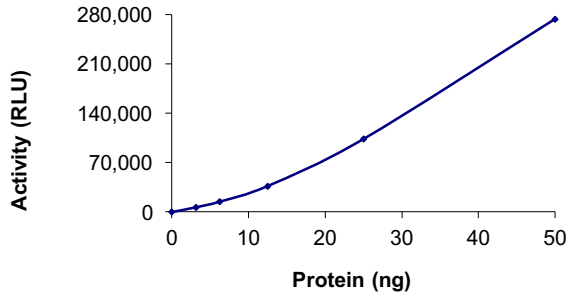
Scientific Background

ALK or CD246 is a receptor tyrosine kinase, which belongs to the insulin receptor superfamily. CD246 plays an important role in the development of the brain and exerts its effects on specific neurons in the nervous system. ALK-positive neoplasms represent a distinct entity because the morphology of the tumors is often neither anaplastic nor large cell and the tumors should be referred to as ALK lymphomas (1). CD246 has been found to be rearranged, mutated, or amplified in a series of tumours including anaplastic large cell lymphomas, neuroblastoma, and non-small cell lung cancer. CD246 act as a driver of inflammation. TPM1-ALK has been identified in the patients with peritoneal mesotheliomas (2).

References

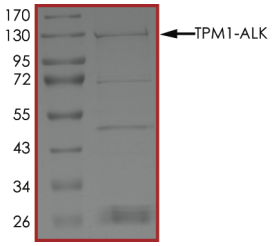
1. Benharroch, D.et.al: ALK-positive lymphoma: a single disease with a broad spectrum of morphology. Blood 91: 2076-2084, 1998.
2. Hung YP. et al: Identification of ALK Rearrangements in Malignant Peritoneal Mesothelioma. JAMA Oncology. 2018 Feb 1; 4(2): 235-238.

Specific Activity



The specific activity of TPM1-ALK was determined to be **22 nmol /min/mg** as per activity assay protocol.

Purity



The purity of TPM1-ALK was determined to be **>70%** by densitometry, approx. MW **125kDa**.

TPM1-ALK, Active

Human recombinant protein expressed in Sf9 cells

Catalog #	A19-19LG
Specific Activity	22 nmol/min/mg
Lot #	M2982-7
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.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: A19-19LG)

Active TPM1-ALK (0.05 μg/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 TPM1-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 $MnCl_2$ 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 (KKKSPGEYVNIEFG) diluted in distilled H2O to a final concentration of 1mg/ml.

Cofactor: 2.5M $MnCl_2$ (Catalog #: M40-09-25)

Diluted to a working concentration of 0.1M in distilled H_2O.

Assay Protocol

The TPM1-ALK assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the TPM1-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 Active TPM1-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 TPM1-ALK (Catalog # A19-19LG).
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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