

## EML4-ALK (Eex20Aex20), Active

Human recombinant protein expressed in Sf9 cells

Catalog # A19-19HG

Lot # M2622-8

### Product Description

Recombinant human fusion protein EML4 (1-580a.a., exons1-20) - ALK (1058-end, exons20-29) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number of EML4 is [NM\\_019063](#), and ALK is [NM\\_004304](#).

### Gene Aliases

EML4: C2orf2, EMAPL4

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  $-70^{\circ}\text{C}$ . 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.

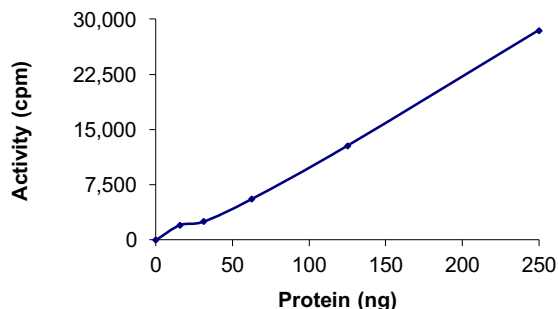
### Scientific Background

ALK or CD246 is a receptor tyrosine kinase, which belongs to the insulin receptor superfamily which comprises an extracellular domain, a hydrophobic stretch corresponding to a single pass transmembrane region, and an intracellular kinase domain. 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. EML4-ALK mutations in lung cancer confer resistance to ALK inhibitors (2).

### References

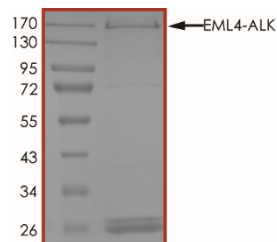
1. Benharroch, D. et.al: ALK-positive lymphoma: a single disease with a broad spectrum of morphology. *Blood* 91: 2076-2084, 1998.
2. Choi, Y. L. et.al: EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. *New Eng. J. Med.* 363: 1734-1739, 2010.

### Specific Activity



The specific activity of EML4-ALK (Eex20Aex20) was determined to be **5.7 nmol/min/mg** as per activity assay protocol.

### Purity



The purity of EML4-ALK (Eex20Aex20) was determined to be **>70%** by densitometry, approx. MW **165 kDa**.

## EML4-ALK (Eex20Aex20), Active

Human recombinant protein expressed in Sf9 cells

Catalog #	A19-19HG
Specific Activity	5.7 nmol/min/mg
Lot #	M2622-8
Purity	>70%
Concentration	0.05 µg/µl
Stability	1yr at $-70^{\circ}\text{C}$ from date of shipment
Storage & Shipping	Store product at $-70^{\circ}\text{C}$ . 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 #: A19-19HG)

Active EML4-ALK (Eex20Aex20) (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 EML4-ALK (Eex20Aex20) 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 MgCl<sub>2</sub>, 12.5mM MnCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>33</sup>P]-ATP Assay Cocktail

Prepare 250µM [<sup>33</sup>P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [<sup>33</sup>P]-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 (KKKSPGEYVNIEFG) diluted in distilled H<sub>2</sub>O to a final concentration of 1 mg/ml.

## Assay Protocol

- Step 1.** Thaw [<sup>33</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active EML4-ALK (Eex20Aex20), 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 EML4-ALK (Eex20Aex20) (Catalog # A19-19HG)
  - Component 2.** 5µl of 1 mg/ml stock solution of substrate (Catalog #I15-58)
  - Component 3.** 5µl distilled H<sub>2</sub>O (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 H<sub>2</sub>O.
- Step 5.** Initiate the reaction by the addition of 5µl [<sup>33</sup>P]-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 H<sub>2</sub>O) 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 [<sup>33</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [<sup>33</sup>P]-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 <sup>33</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg)]\*[(Reaction Volume) / (Spot Volume)]

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