Human ALK/CD246 Antibody

Antigen Affinity-purified Polyclonal Sheep IgG
Catalog Number: AF4210

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human ALK/CD246 in direct ELISAs and Western blots.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	Chinese hamster ovary cell line CHO-derived recombinant human ALK/CD246 Val19-Ser1038 Accession # Q9UM73
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

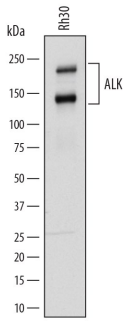
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Simple Western	5 µg/mL	SH-SY5Y human neuroblastoma cells

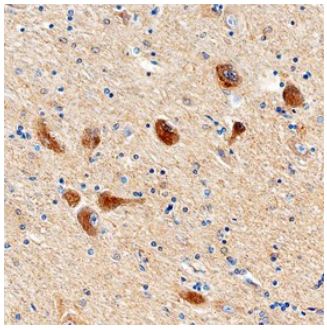
DATA

Western Blot



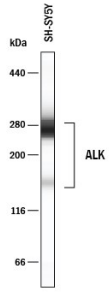
Detection of Human ALK/CD246 by Western Blot. Western blot shows lysates of Rh30 human rhabdomyosarcoma cell line. PVDF membrane was probed with 0.5 µg/mL of Sheep Anti-Human ALK/CD246 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4210) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). Specific bands were detected for ALK/CD246 at approximately 220 and 140 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



ALK/CD246 in Human Brain. ALK/CD246 was detected in immersion fixed paraffin-embedded sections of human brain (hypothalamus) using Sheep Anti-Human ALK/CD246 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4210) at 3 µg/mL overnight at 4 °C. Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific staining was localized to neuronal cell bodies. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Simple Western



Detection of Human ALK/CD246 by Simple Western™. Simple Western lane view shows lysates of SH-SY5Y human neuroblastoma cells, loaded at 0.2 mg/mL. Specific bands were detected for ALK/CD246 at approximately 158 and 266 kDa (as indicated) using 5 µg/mL of Sheep Anti-Human ALK/CD246 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4210) followed by 1:50 dilution of HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). This experiment was conducted under reducing conditions and using the 66-440kDa separation system.

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Catalog Number: AF4210

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.2 mg/mL.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

ALK (Anaplastic Lymphoma Kinase; also CD246) is a 200-220 kDa member of the Insulin receptor subfamily, tyrosine kinase family, protein kinase superfamily of proteins. It shows restricted expression, being limited to select sympathetic and sensory neurons, endothelial cells and tumor cells. Although activation of ALK appears to induce cell proliferation, the exact ligand(s) for ALK is unknown. In a temporally-regulated context, midkine has been suggested to bind to ALK, and ionic Zn has been reported to activate ALK cytoplasmically. Mature human ALK is a 1602 amino acid (aa) type I transmembrane glycoprotein (SwissProt #:Q9UM73). It contains a 1020 aa extracellular region (aa 19-1038) that contains one protein-protein MAM domain (aa 264-427), an LDL receptor class A region (aa 437-473), a second MAM domain (aa 480-631) and one utilized phosphorylation site at Ser211. This is accompanied by a long 561 aa cytoplasmic domain that possesses a protein kinase domain (aa 1116-1392) plus eleven utilized Tyr phosphorylation sites. There is one potential splice variant that contains a four aa insert after Glu549. There is also an 80 kDa soluble product that arises from cleavage of the extracellular domain. Although a third 140 kDa product is frequently seen in SDS-Page, its structural basis is unclear. Almost 20 distinct ALK fusion products arise from gene translocations involving multiple intracellular molecules. These seem to involve blocks of aa starting between aa 1050-1060 and continuing to aa 1620. Over aa 19-1038, human ALK shares 88% aa sequence identity with mouse ALK.

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