

Store at
-20°C

ALK (D5F3®) XP® Rabbit mAb

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#3633

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UniProt ID #Q9UM73

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For Research Use Only. Not For Use In Diagnostic Procedures.

| Applications | Species Cross-Reactivity* | Molecular Wt. | Isotype |
|-------------------------------|---------------------------|---|--------------|
| W, IP, IHC-P, F Endogenous | H | 220 (ALK), 80 (NPM-ALK), 117 (EML4-ALK v1), 86 (EML4-ALK v3) kDa | Rabbit IgG** |

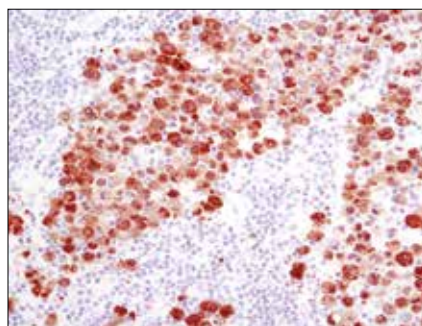
Background: Anaplastic lymphoma kinase (ALK) is a tyrosine kinase receptor for pleiotrophin (PTN), a growth factor involved in embryonic brain development (1-3). In ALK-expressing cells, PTN induces phosphorylation of both ALK and the downstream effectors IRS-1, Shc, PLC γ , and PI3 kinase (1). ALK was originally discovered as a nucleophosmin (NPM)-ALK fusion protein produced by a translocation (4). Investigators have found that the NPM-ALK fusion protein is a constitutively active, oncogenic tyrosine kinase associated with anaplastic lymphoma (4). Research literature suggests that activation of PLC γ by NPM-ALK may be a crucial step for its mitogenic activity and involved in the pathogenesis of anaplastic lymphomas (5).

A distinct ALK oncogenic fusion protein involving ALK and echinoderm microtubule-associated protein like 4 (EML4) has been described in the research literature from a non-small cell lung cancer (NSCLC) cell line, with corresponding fusion transcripts present in some cases of lung adenocarcinoma. The short, amino-terminal region of the microtubule-associated protein EML4 is fused to the kinase domain of ALK (6-8).

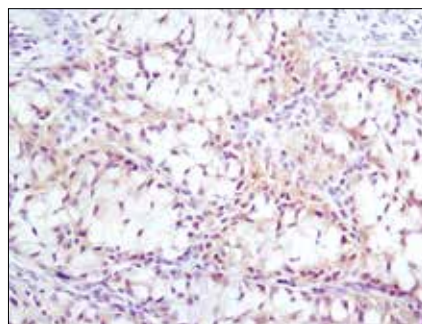
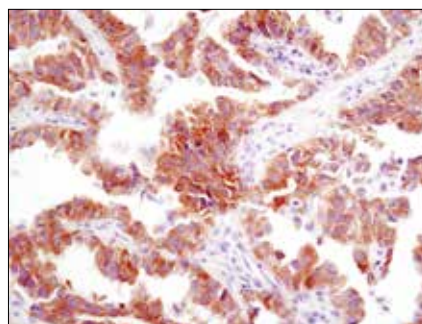
Investigators have identified ALK translocations with other fusion partners, such as TRK-fused gene (TFG) and KIF5B, which have also been associated with NSCLC (6,7). In particular, the EML4-ALK fusion protein has been found in 3-7% of NSCLC samples (6-14).

Specificity/Sensitivity: ALK (D5F3®) XP® Rabbit mAb detects endogenous levels of total ALK protein as well as ALK fusion proteins, such as EML4-ALK variants and NPM-ALK, even at low levels. This antibody does not cross-react with other family members.

Source/Purification: Monoclonal antibody is produced by immunizing animals with recombinant protein corresponding to residues in the carboxy terminus of human ALK.



Immunohistochemical analysis of paraffin-embedded human lung carcinoma using ALK (D5F3®) XP® Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human lung carcinoma with high (left) and low levels (right) of ALK expression using ALK (D5F3®) XP® Rabbit mAb.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μ g/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

*Species cross-reactivity is determined by western blot.

**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

| | |
|---|--|
| Western blotting | 1:2000 |
| Immunoprecipitation | 1:100 |
| Immunohistochemistry (Paraffin) | 1:250† |
| Unmasking buffer: | EDTA |
| Antibody diluent: | SignalStain® Antibody Diluent #8112 |
| Detection reagent: | SignalStain® Boost (HRP, Rabbit) #8114 |
| †Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent. | |
| Flow Cytometry | 1:400 |

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

Background References:

- (1) Stoica, G.E. et al. (2001) *J Biol Chem* 276, 16772-9.
- (2) Iwahara, T. et al. (1997) *Oncogene* 14, 439-49.
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- (4) Morris, S.W. et al. (1994) *Science* 263, 1281-4.
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- (6) Rikova, K. et al. (2007) *Cell* 131, 1190-203.
- (7) Takeuchi, K. et al. (2008) *Clin Cancer Res* 14, 6618-24.
- (8) Soda, M. et al. (2007) *Nature* 448, 561-6.
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- (12) Rodig, S.J. et al. (2009) *Clin Cancer Res* 15, 5216-23.
- (13) Mino-Kenudson, M. et al. (2010) *Clin Cancer Res* 16, 1561-71.
- (14) Kwak, E.L. et al. (2010) *N Engl J Med* 363, 1693-703.

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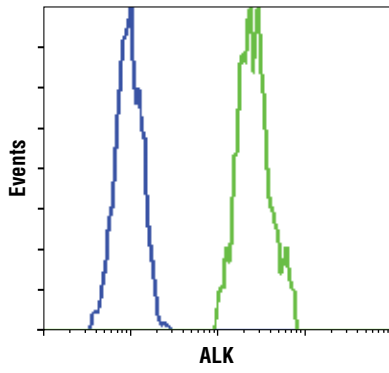
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IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

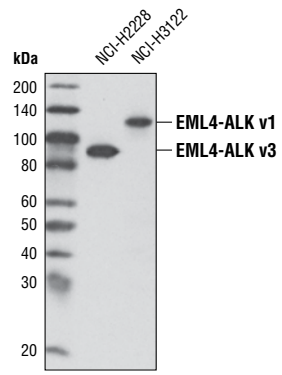
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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide Species Cross-Reactivity: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



Flow cytometric analysis of DU 145 cells (blue) and KARPAS-299 cells (green) using ALK (D5F3[®]) XP[®] Rabbit mAb. Cell Line Source: Dr Abraham Karpas at the University of Cambridge.



Western blot analysis of extracts from NCI-H2228 and NCI-H3122 cells using ALK (D5F3[®]) XP[®] Rabbit mAb. Variants denoting fusions of different EML4 exons (v1 or v3) are indicated.