Store at -20°C

# #3633 ALK (D5F3) XP® Rabbit mAb

Cell Signaling TECHNOLOGY®

☐ Small (250 slides) 100 µl

☐ Petite (100 slides) 40 µl

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rev. 01/07/13

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

**Entrez-Gene ID** #238
**Swiss-Prot Acc.** #Q9UM73

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

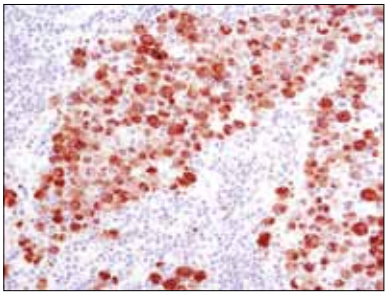
**Background:** Anaplastic lymphoma kinase (ALK) is a tyrosine kinase receptor for pleiotrophin (PTN), a growth factor involved in embryonic brain development (1-3). In ALK-expressing cells, PTN induces phosphorylation of both ALK and the downstream effectors IRS-1, Shc, PLCγ, and PI3 kinase (1). ALK 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 PLCγ 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).

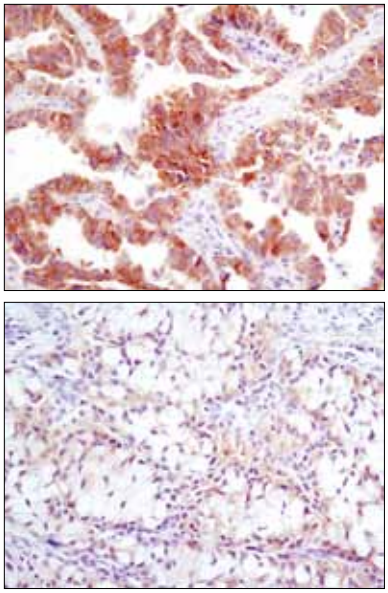
Investigators have identified ALK translocations with other fusion partners, such as TRK-fused gene (TFG) and KIF5B, which have also been associated with NSCLC (6,7). In particular, the EML4-ALK fusion protein has been found in 3-7% of NSCLC samples (6-14).

**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 anaplastic large cell lymphoma (ALCL) using ALK (D5F3) XP® Rabbit mAb.*



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

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/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.*

| | |
|---|---|
| Immunofluorescence (IF-IC) | 1:200 |
| Flow Cytometry | 1:400 |

**Note: Due to strong signal the antibody may need further dilution for western blotting.**

**For product specific protocols please see the web page for this product at www.cellsignal.com.**

**Please visit www.cellsignal.com for a complete listing of recommended complementary products.**

**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 Key:** **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

**Species Cross-Reactivity Key:** **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 **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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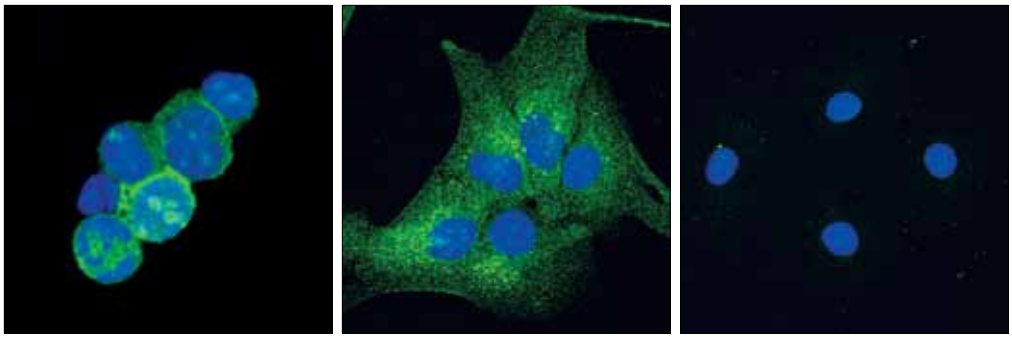
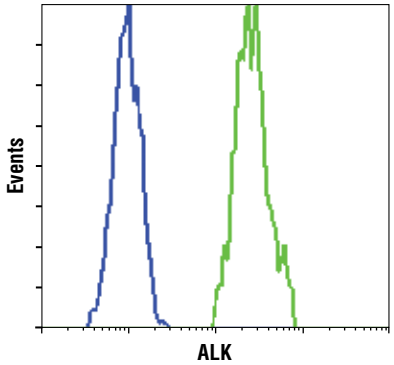
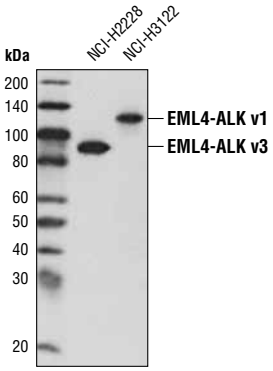


*Confocal immunofluorescent analysis of KARPAS-299 cells (left), NCI-H2228 cells (center) and DU 145 cells (right), using ALK (D5F3) XP® Rabbit mAb (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).*




*Flow cytometric analysis of DU 145 cells (blue) and KARPAS-299 cells (green) using ALK (D5F3) XP® Rabbit mAb.*




*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.*

**Background References:**


(1) Stoica, G.E. et al. (2001) *J Biol Chem* 276, 16772-9.

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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.

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(12) Rodig, S.J. et al. (2009) *Clin Cancer Res* 15, 5216-23.

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