Store at -20°C

ALK (D5F3®) XP® Rabbit mAb

#3633

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rev. 01/13/16

Entrez-Gene ID #238
UniProt ID #Q9UM73

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

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**

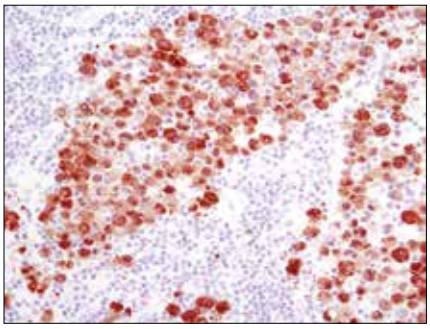
Background: Anaplastic lymphoma kinase (ALK) is a tyrosine kinase receptor for pleiotrophin (PTN), a growth factor involved in embryonic brain development (1-3). In ALK-expressing cells, PTN induces phosphorylation of both ALK and the downstream effectors IRS-1, Shc, PLCγ, and PI3 kinase (1). ALK was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

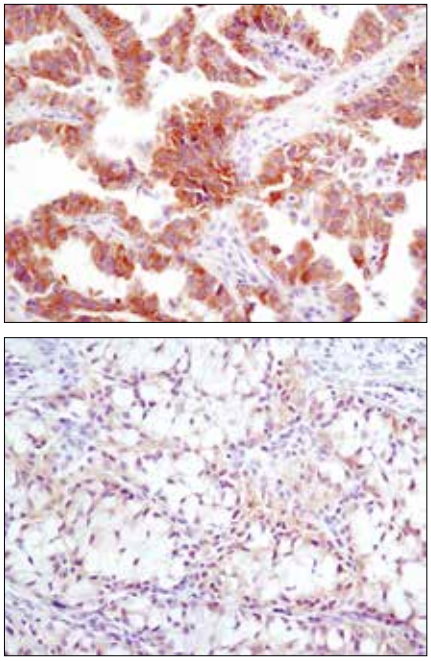
Investigators have identified ALK translocations with other fusion partners, such as TRK-fused gene (TFG) and KIF5B, which have also been associated with NSCLC (6,7). In particular, the EML4-ALK fusion protein has been found in 3-7% of NSCLC samples (6-14).

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 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 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

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.

(3) Morris, S.W. et al. (1997) *Oncogene* 14, 2175-88.

(4) Morris, S.W. et al. (1994) *Science* 263, 1281-4.

(5) Bai, R.Y. et al. (1998) *Mol Cell Biol* 18, 6951-61.

(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.

(9) Takeuchi, K. et al. (2009) *Clin Cancer Res* 15, 3143-9.

(10) Palmer, R.H. et al. (2009) *Biochem J* 420, 345-61.

(11) Horn, L. and Pao, W. (2009) *J Clin Oncol* 27, 4232-5.

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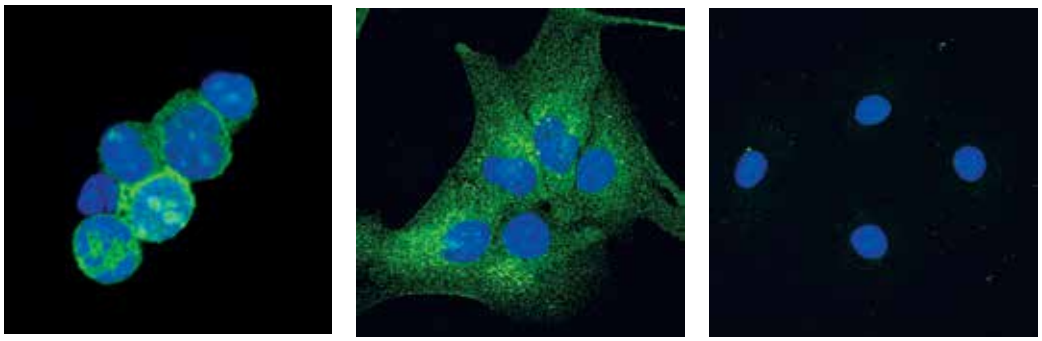
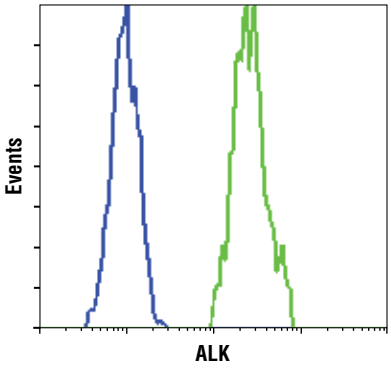
IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

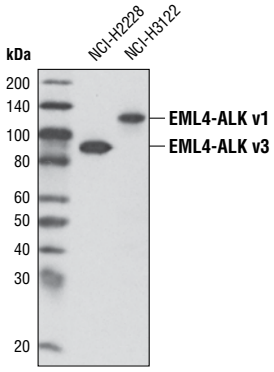
Cell Signaling TECHNOLOGY®



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). Cell Line Source: Dr Abraham Karpas at the University of Cambridge.



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.