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**1**00 μl (10 western blots)

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

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype	
W, IP Endogenous	H, (M, R)	220 (ALK), 80 (NPM-ALK) kDa	Rabbit IgG**	

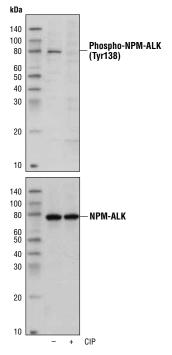
Background: Anaplastic lymphoma kinase (ALK) is a tyrosine kinase receptor for pleiotrophin (PTN), a growth factor involved in embryonic brain development (1-3). In ALKexpressing cells, PTN induces phosphorylation of both ALK and the downstream effectors IRS-1, Shc, PLCy, and PI3 kinase (1). ALK was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

Phosphorylation of ALK at Tyr1078 was identified at Cell Signaling Technology (CST) using PhosphoScan®, CST's LC-MS/MS platform for phosphorylation site discovery. Phosphorylation of ALK at Tyr1078 was observed in select carcinoma cell lines and in tumors.

Specificity/Sensitivity: Phospho-ALK (Tyr1078) (D28B4) Rabbit mAb recognizes endogenous levels of ALK only when phosphorylated at Tyr1078, which is equivalent to Tyr138 of NPM-ALK. This antibody may cross-react weakly with other overexpressed phospho-tyrosine kinases such as EGFR and Src.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Tyr1078 of human ALK protein.



Western blot analysis of extracts from KARPAS-299 cells, untreated (-) or treated with calf intestinal phosphatase (CIP; +), using Phospho-ALK (Tyr1078) (D28B4) Rabbit mAb (upper) or ALK (C26G7) Rabbit mAb #3333 (lower). Cell Line Source: Dr Abraham Karpas at the University of Cambridge.

#### Entrez-Gene ID #238 UniProt ID #Q9UM73

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:**

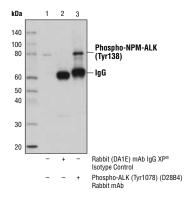
Western blotting 1:1000 Immunoprecipitation 1:100

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.

#### **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.



Immunoprecipitation of phospho-NPM-ALK(Tyr138) from KARPAS-299 cell extracts, using Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (Jane 2) or Phospho-ALK (Tyr1078) (D28B4) Rabbit mAb (lane 3). Lane 1 is 10% input. Western blot analysis was performed using Phospho-ALK (Tyr1078) (D28B4) Rabbit mAb. Cell Line Source: Dr Abraham Karpas at the University of Cambridge.

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