Store at –20°C

#3348

Phospho-ALK (Tyr1586) (3B4) Rabbit mAb

Cell Signaling TECHNOLOGY®

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Entrez-Gene ID #238
UniProt ID #Q9UM73

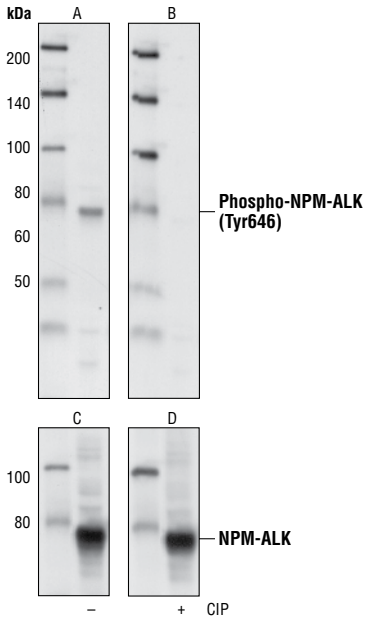
Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W Endogenous	H	80 kDa NPM-ALK fusion; 220 kDa ALK	Rabbit IgG**

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 an NPM (nucleophosmin)-ALK fusion protein produced by a translocation (4). The NPM-ALK fusion protein is a constitutively active, oncogenic tyrosine kinase associated with anaplastic lymphoma (4). Activation of PLCγ by NPM-ALK has been suggested to be a crucial step for its mitogenic activity and may be important in the pathogenesis of anaplastic lymphomas (5).

A distinct ALK oncogenic fusion protein involving ALK and EML4 has been described from a non-small cell lung cancer 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 (6,7).

Specificity/Sensitivity: Phospho-ALK (Tyr1586) (3B4) Rabbit mAb detects ALK only when phosphorylated at Tyr1586 (equivalent to Tyr646 of NPM-ALK). This antibody may cross-react with other activated protein tyrosine kinases including EGFR.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Tyr1586 of human ALK.



Western blot analysis of Sup-M2 cell lysate, using Phospho-ALK (Tyr1586) (3B4) Rabbit mAb (A and B), and ALK Antibody #3342 (C and D).The phospho-specificity of this antibody was characterized by treating the membrane with calf intestinal alkaline phosphatase (CIP) (B and D) after western transfer.

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

For application 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 companion 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.

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.

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 **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.