

Store at
-20°C

ALK Activation Antibody Sampler Kit



#8336

1 Kit (9 x 20 µl)

Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

Orders: 877-616-2355 (U.S.)
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Entrez-Gene ID #238, 207,
UniProt ID #Q9UM73,

rev. 06/16

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-ALK (Tyr1586) (3B4) Rabbit mAb	3348	20 µl	80, 220 kDa	Rabbit IgG
Phospho-Jak2 (Tyr1007) (D15E2) Rabbit mAb	4406	20 µl	125 kDa	Rabbit IgG
Phospho-Jak3 (Tyr980/981) (D44E3) Rabbit mAb	5031	20 µl	115 kDa	Rabbit IgG
Phospho-Stat3 (Tyr705) (D3A7) XP® Rabbit mAb	9145	20 µl	79, 86 kDa	Rabbit IgG
Phospho-Stat5 (Tyr694) (D47E7) XP® Rabbit mAb	4322	20 µl	90 kDa	Rabbit IgG
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb	4370	20 µl	44, 42 kDa	Rabbit IgG
Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb	4060	20 µl	60 kDa	Rabbit IgG
Phospho-Src Family (Tyr416) (D49G4) Rabbit mAb	6943	20 µl	60 kDa	Rabbit IgG
Phospho-PLCγ1 (Tyr783) (D6M9S) Rabbit mAb	14008	20 µl	155 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

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

Description: The ALK Activation Antibody Sampler Kit provides an economical means to evaluate the activation status of multiple members of the ALK pathway, including phosphorylated ALK, Jak2, Jak3, Stat3, Stat5, PLCγ1, Akt, Src, and p44/42 MAPK. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

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).

Specificity/Sensitivity: Each antibody in the ALK Activation Antibody Sampler Kit recognizes the phosphorylated form of its specific target. Phospho-ALK (Tyr1586) (3B4) Rabbit mAb may cross-react with other activated protein tyrosine kinases including EGFR. Phospho-Jak2 (Tyr1007) (D15E2) Rabbit mAb may cross react with phosphorylated Jak3 and Tyk2. Phospho-Src Family (Tyr416) (D49G4) Rabbit mAb may cross react with overexpressed phosphorylated RTKs.

Source/Purification: Rabbit monoclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Tyr1586 of human ALK, Tyr1007 of human Jak2, Tyr980/981 of human and mouse Jak3, Tyr705 of mouse Stat3, Tyr694 of human Stat5a, Thr202/Tyr204 of human p44 MAP kinase, Ser473 of human Akt, Tyr416 of human Src, or Tyr783 of human PLCγ1 protein.

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.

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 antibodies.*

Recommended Antibody Dilutions:

Western blotting 1:1000

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

U. S. Patent No. 5,675,063
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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.

Western Immunoblotting Protocol

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

NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

- 20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH₂O, mix.
- 10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH₂O, mix.
- 1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723)
Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH₂O.
- 10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH₂O, mix.
- 10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH₂O, mix.
- 10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH₂O, mix.
- Nonfat Dry Milk:** (#9999)
- Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
- Wash Buffer:** (#9997) 1X TBST
- Bovine Serum Albumin (BSA):** (#9998)
- Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
- Biotinylated Protein Ladder Detection Pack:** (#7727)
- Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
- Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
- Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
- Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
- Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
- Microcentrifuge for 5 min.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
- Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
- Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

- Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 min each with 15 ml of TBST.
- Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
- Wash three times for 5 min each with 15 ml of TBST.
- Proceed with detection (Section D).

D. Detection of Proteins

- Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time.
NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.

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