Store at -20°C

#9328

c-Oncogene Antibody Sampler Kit

☑ 1 Kit
(9 x 40 µl)

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 05/18/11

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
c-Abl Antibody	2862	40 µl	135, 210 kDa	Rabbit IgG
c-Fos (9F6) Rabbit mAb	2250	40 µl	62 kDa	Rabbit IgG
c-Jun (60A8) Rabbit mAb	9165	40 µl	43, 48 kDa	Rabbit IgG
c-Kit (D13A2) Rabbit mAb	3074	40 µl	120, 145 kDa	Rabbit IgG
c-Myc (D84C12) XP® Rabbit mAb	5605	40 µl	57-65 kDa	Rabbit IgG
c-Raf Antibody	9422	40 µl	65-75 kDa	Rabbit IgG
Ras (27H5) Rabbit mAb	3339	40 µl	21 kDa	Rabbit IgG
c-Rel Antibody	4727	40 µl	78 kDa	Rabbit IgG
Src (36D10) Rabbit mAb	2109	40 µl	60 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 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 a complete listing of recommended companion products.

Description: The c-Oncogene Antibody Sampler Kit provides an economical means of evaluating total levels of various oncogenic proteins. The kit contains enough primary and secondary antibodies to perform four western blot experiments.

Background: The regulation of cell growth, differentiation and programmed death is coordinated by several sets of proteins that comprise essential signal transduction pathways. Many of these key regulatory proteins are encoded by proto-oncogenes, which can be activated (altered) to change the typical cell program to one of abnormal cell growth and unregulated development. Proteins encoded by proto-oncogenes include growth factors and other ligands, receptor proteins, tyrosine kinases, various regulatory proteins (i.e. GTPases) and transcription factors. Together these proteins comprise the basic elements of cell signaling pathways; altered expression or mutation of one or more of these components can lead to oncogenic growth (reviewed in 1).

Non-receptor (i.e. cytoplasmic, nuclear) tyrosine kinases such as c-Abl and Src play key roles in the regulation of cell proliferation, differentiation, apoptosis, cell adhesion and stress responses (2,3). Alteration of the corresponding c-Abl and Src proto-oncogenes is associated with oncogenesis; Abl1-BCR gene translocations result in chronic myelogenous leukemia (CML) while constitutively active Src is seen in some patients with colon cancer and altered Src expression is seen in a wide array of cancers (2,4). Regulation of Raf tyrosine kinase by Ras GTPase controls downstream kinases in the MEK/MAPK signaling pathway (5). Activation of the Ras and Raf proto-oncogenes are common in human cancers and both proteins are seen as potential therapeutic targets (6). The receptor tyrosine kinase c-Kit plays a critical role in activation and growth of hematopoietic stem cells (7); mutations that inhibit c-Kit kinase activity are associated with a variety of developmental disorders while mutations producing constitutively active c-Kit can result in mastocytosis and gastrointestinal stromal tumors (8). The alteration of key transcription factors such as c-Fos, c-Jun, c-Myc and c-Rel that are normally responsible for regulating cell and tissue growth, differentiation and the inflammation/immune response, can also result in unregulated, oncogenic cell growth (9-12).

Specificity/Sensitivity: Unless otherwise indicated, each antibody in the c-Oncogene Antibody Sampler Kit detects endogenous levels of total target protein and does not cross-react with related proteins. c-Jun (60A8) Rabbit mAb detects endogenous levels of total c-Jun protein, regardless of phosphorylation state. Ras (27H5) Rabbit mAb detects endogenous levels of total K-Ras, H-Ras and N-Ras proteins. Src (36D10) Rabbit mAb detects endogenous levels of Src proteins and may cross-react with other Src family members. The c-Myc (D84C12) Rabbit mAb detects endogenous levels of total c-Myc protein.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues near the carboxy terminus of human c-Rel, a region surrounding Pro302 of human c-Raf, and corresponding to the sequence close to the carboxy-terminus of human c-Abl. Antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibody is produced by immunizing animals with synthetic peptides corresponding to residues near the carboxy terminus of human Src, residues near the amino terminus of human K-Ras, from the amino-terminal sequence of human c-Jun, from the sequence of human c-Fos, residues near the amino terminus of c-Myc and corresponding to the residues surrounding Tyr703 of human c-Kit.

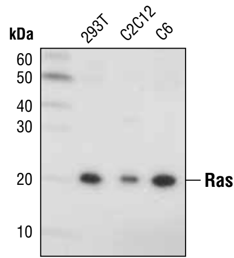
Background References:

(1) Croce, C.M. (2008) *N Engl J Med* 358, 502–11.
(2) Wang, J.Y. (2000) *Oncogene* 19, 5643–50.
(3) Thomas, S.M. and Brugge, J.S. (1997) *Annu Rev Cell Dev Biol* 13, 513–609.
(4) Dehm, S.M. and Bonham, K. (2004) *Biochem Cell Biol* 82, 263–74.
(5) Avruch, J. et al. (1994) *Trends Biochem Sci* 19, 279–83.
(6) Stites, E.C. et al. (2007) *Science* 318, 463–7.
(7) Gommerman, J.L. et al. (1997) *J Biol Chem* 272, 30519–25.
(8) Nocka, K. et al. (1990) *EMBO J* 9, 1805–13.
(9) Milde-Langosch, K. (2005) *Eur J Cancer* 41, 2449–61.
(10) Shaulian, E. and Karin, M. (2002) *Nat Cell Biol* 4, E131–6.
(11) Yokota, J. et al. (1986) *Science* 231, 261–5.
(12) Rayet, B. and Gélinas, C. (1999) *Oncogene* 18, 6938–47.

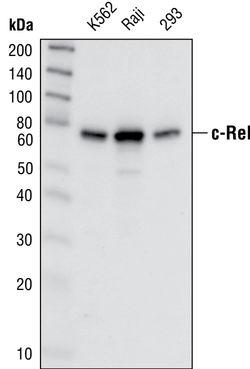
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebra fish **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.

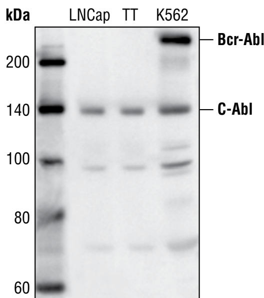
© 2011 Cell Signaling Technology, Inc.
Selected rabbit monoclonal antibodies are produced under license (granting certain rights including those under U. S. Patent No. 5,675,063 and/or U.S.S.N. 11/476,277) from Epitomics, Inc.



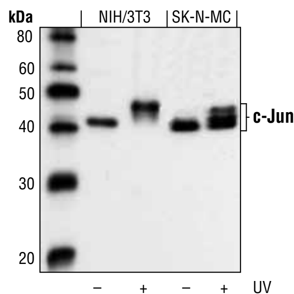
*Western blot analysis of extracts from 293T, C2C12 and C6 cells using **Ras (27H5) Rabbit mAb #3339**.*



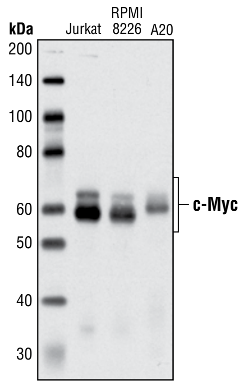
*Western blot analysis of extracts from K562, Raji and 293 cells using **c-Rel Antibody #4727**.*



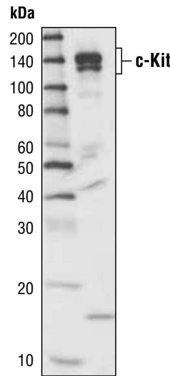
*Western blot analysis of extracts from LNCap, TT and K562, using **c-Abl Antibody #2862**.*



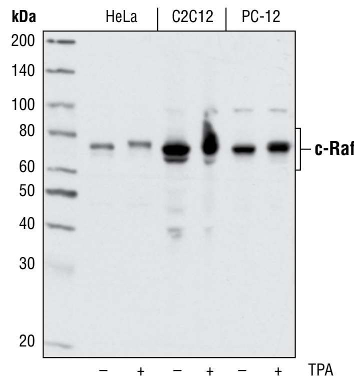
*Western blot analysis of extracts from NIH/3T3 and SK-N-MC cells, untreated or UV-treated, using **c-Jun (60A8) Rabbit mAb #9165**.*



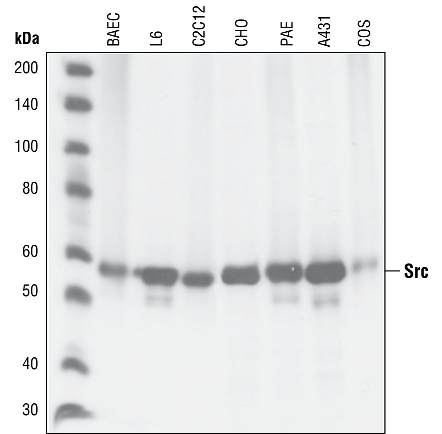
*Western blot analysis of extracts from various cell lines using **c-Myc (D84C12) XP® Rabbit mAb #5605**.*



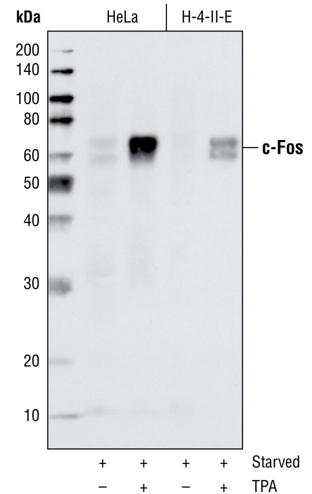
*Western blot analysis of extracts from H526 cells using **c-Kit (D132A) Rabbit mAb #3074**.*



*Western blot analysis of extracts from HeLa, C2C12 or PC-12 cells, untreated or TPA-treated (200 nM for 30 minutes), using **c-Raf Antibody #9422**.*



*Western blot analysis of extracts from various cell lines using **Src (36D10) Rabbit mAb #2109**.*



*Western blot analysis of extracts from HeLa and H-4-II-E cells, serum-starved overnight and TPA-stimulated for 4 hours, using **c-Fos (9F6) Rabbit mAb #2250**.*

© 2008 Cell Signaling Technology, Inc.

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

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.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. **1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
10. **Phototope®-HRP Western Blot Detection System** #7071**:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.

© 2008 Cell Signaling Technology, Inc.