#9328 Store at -20°C

c-Oncogene Antibody Sampler Kit

1 Kit
(9 x 20 µl)

Cell Signaling TECHNOLOGY®

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
c-Abl Antibody	2862	20 µl	135, 210 kDa	Rabbit IgG
c-Fos Antibody	4384	20 µl	62 kDa	Rabbit IgG
c-Jun (60A8) Rabbit mAb	9165	20 µl	43, 48 kDa	Rabbit IgG
c-Kit (D13A2) Rabbit mAb	3074	20 µl	120, 145 kDa	Rabbit IgG
c-Myc (D84C12) Rabbit mAb	5605	20 µl	57-65 kDa	Rabbit IgG
c-Raf Antibody	9422	20 µl	65-75 kDa	Rabbit IgG
Ras (27H5) Rabbit mAb	3339	20 µl	21 kDa	Rabbit IgG
c-Rel (D4Y6M) Rabbit mAb	12707	20 µl	68-78 kDa	Rabbit IgG
Src (32G6) Rabbit mAb	2123	20 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 validation data and 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 includes enough antibody to perform two western blot experiments with each primary antibody.

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 (32G6) Rabbit mAb detects endogenous levels of Src proteins and does not 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, residues near the carboxy-terminus of human c-Fos, 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 a recombinant fusion protein corresponding to residues 1-110 of human Src, residues near the amino terminus of human K-Ras, from the amino-terminal sequence of human c-Jun, 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.
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(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.

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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.

#9328

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.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **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 dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **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 dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **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.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **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.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

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 for a 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 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. 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.
8. Electrotransfer to nitrocellulose membrane (#12369).

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.

I. Membrane Blocking

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

II. Primary Antibody Incubation

1. 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.
2. Wash three times for 5 min each with 15 ml of TBST.
3. 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.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. 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.
2. 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.