Store at -20°C

Phospho-EGF Receptor Pathway Antibody Sampler Kit

#9789

1 Kit (8 x 20 µl)

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

Orders: 877-616-2355 (U.S.)
orders@cellsignal.com

Entrez-Gene ID #207, 208,
UniProt ID #P31749,

rev. 06/16

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

Products Included	Product #	Quantity	Mol. Wt.	Source
Phospho-EGF Receptor (Tyr1068) (D7A5) XP® Rabbit mAb	3777	20 µl	175 kDa	Rabbit IgG
Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb	4060	20 µl	60 kDa	Rabbit IgG
Phospho-c-Cbl (Tyr700) (D16D7) Rabbit mAb	8869	20 µl	120 kDa	Rabbit IgG
Phospho-Gab1 (Tyr627) (C32H2) Rabbit mAb	3233	20 µl	110 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-PLCγ1 (Tyr783) (D6M9S) Rabbit mAb	14008	20 µl	155 kDa	Rabbit IgG
Phospho-Shc (Tyr239/240) Antibody	2434	20 µl	50, 55, 70 kDa	Rabbit IgG
Phospho-Stat5 (Tyr694) (D47E7) XP® Rabbit mAb	4322	20 µl	90 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

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.

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

Description: The Phospho-EGF Receptor Pathway Sampler Kit provides an economical means to evaluate the activation status of multiple members of the EGF receptor pathway, including phosphorylated EGF receptor, Stat5, c-Cbl, Shc, Gab1, PLCγ1, Akt and p44/42 MAPK. The kit includes enough antibody to perform two western blot experiments with each primary antibody..

Background: The epidermal growth factor (EGF) receptor is a transmembrane tyrosine kinase that belongs to the HER/ErbB protein family. Ligand binding results in receptor dimerization, autophosphorylation, activation of downstream signaling, internalization, and lysosomal degradation (1,2). Phosphorylation of EGF receptor (EGFR) at Tyr845 in the kinase domain is implicated in stabilizing the activation loop, maintaining the active state enzyme, and providing a binding surface for substrate proteins (3,4). c-Src is involved in phosphorylation of EGFR at Tyr845 (5). The SH2 domain of PLCγ binds at phospho-Tyr992, resulting in activation of PLCγ-mediated downstream signaling (6). Phosphorylation of EGFR at Tyr1045 creates a major docking site for the adaptor protein c-Cbl, leading to receptor ubiquitination and degradation following EGFR activation (7,8). The GRB2 adaptor protein binds activated EGFR at phospho-Tyr1068 (9). A pair of phosphorylated EGFR residues (Tyr1148 and Tyr1173) provide a docking site for the Shc scaffold protein, with both sites involved in MAP kinase signaling activation (2). Phosphorylation of EGFR at specific serine and threonine residues attenuates EGFR kinase activity. EGFR carboxy-terminal residues Ser1046 and Ser1047 are phosphorylated by CaM kinase II; mutation of either of these serines results in upregulated EGFR tyrosine autophosphorylation (10).

Specificity/Sensitivity: Each antibody in the Phospho-EGF Receptor Pathway Sampler Kit recognizes the phosphorylated form of its specific target. Phospho-EGF Receptor (Tyr1068) (D7A5) XP® Rabbit mAb may cross-react weakly with other tyrosine-phosphorylated proteins. Phospho-Gab1 (Tyr627) (C32H2) Rabbit mAb may cross-react with phosphorylated Gab2, Gab3, or activated receptor tyrosine kinases. Phospho-Shc (Tyr239/240) Antibody may cross-react with activated EGF receptor protein.

Source/Purification: Activation state polyclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Tyr239/240 of human Shc. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Rabbit monoclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Tyr700 of c-Cbl, Tyr1068 of human EGF receptor, Tyr694 of Stat5a, Tyr627 of human Gab1, Ser473 of human Akt, Thr202/Tyr204 of human p44 MAP kinase, or Tyr783 of human PLCγ1 protein.

Background References:

(1) Hackel, P.O. et al. (1999) *Curr Opin Cell Biol* 11, 184-9.
(2) Zwick, E. et al. (1999) *Trends Pharmacol Sci* 20, 408-12.
(3) Cooper, J.A. and Howell, B. (1993) *Cell* 73, 1051-4.
(4) Hubbard, S.R. et al. (1994) *Nature* 372, 746-54.
(5) Biscardi, J.S. et al. (1999) *J Biol Chem* 274, 8335-43.
(6) Emlet, D.R. et al. (1997) *J Biol Chem* 272, 4079-86.
(7) Levkowitz, G. et al. (1999) *Mol Cell* 4, 1029-40.
(8) Ettenberg, S.A. et al. (1999) *Oncogene* 18, 1855-66.
(9) Rojas, M. et al. (1996) *J Biol Chem* 271, 27456-61.
(10) Feinmesser, R.L. et al. (1999) *J Biol Chem* 274, 16168-73.

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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.

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.