

#9922 Store at -20°C

# Phospho-EGF Receptor Antibody Sampler Kit

1 Kit  
 (4 x 40 µl)

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

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**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
EGF Receptor (D38B1) XP™ Rabbit mAb	4267	40 µl	175 kDa	Rabbit IgG
Phospho-EGF Receptor (Tyr1068) (D7A5) XP™ Rabbit mAb	3777	40 µl	175 kDa	Rabbit IgG
Phospho-EGF Receptor (Tyr992) Antibody	2235	40 µl	175 kDa	Rabbit IgG
Phospho-EGF Receptor (Tyr1045) Antibody	2237	40 µl	175 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

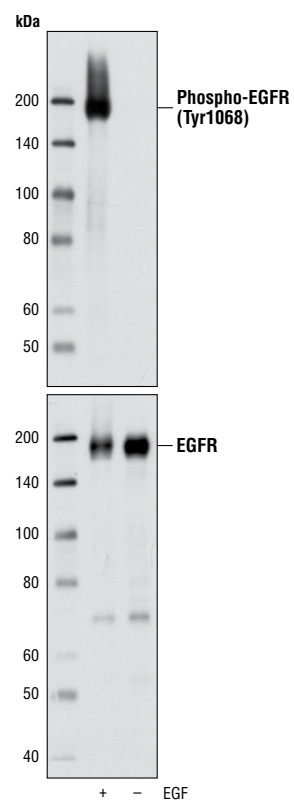
See [www.cellsignaling.com](http://www.cellsignaling.com) for individual component applications, species cross-reactivity, dilutions and additional application protocols.

**Description:** The Phospho-EGF Receptor Antibody Sampler Kit provides an economical means of evaluating the EGF Receptor and several phosphorylation sites that are involved in its activation. The kit contains enough primary and secondary antibodies to perform four Western blot experiments.

**Background:** The epidermal growth factor (EGF) receptor is a 170 kDa transmembrane tyrosine kinase that belongs to the HER/ErbB protein family. Ligand binding results in receptor dimerization, autophosphorylation, activation of downstream signaling 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 c-Cbl, an adaptor protein that leads 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 residues (Tyr1148 and Tyr1173) provides 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 to either of these serines results in upregulated EGFR tyrosine autophosphorylation (10).

**Specificity/Sensitivity:** Each phospho-EGF Receptor antibody recognizes only the phosphorylated form of EGF Receptor at the indicated sites. The control EGF Receptor antibody recognizes both the phosphorylated and non-phosphorylated forms of EGF receptor.

**Source/Purification:** Antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Tyr992, Tyr1045 or Tyr1068 of



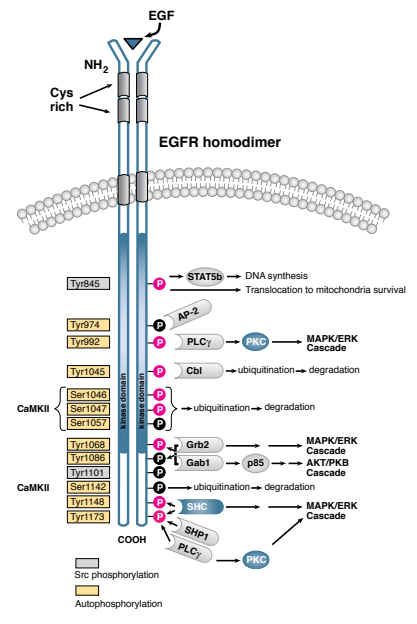
Western blot analysis of extracts of BxPC-3 cells, untreated or EGF-stimulated, using **Phospho-EGF Receptor (Tyr1068) (D7A5) XP™ Rabbit mAb #3777** (upper) and EGF Receptor Antibody #2232 (lower).

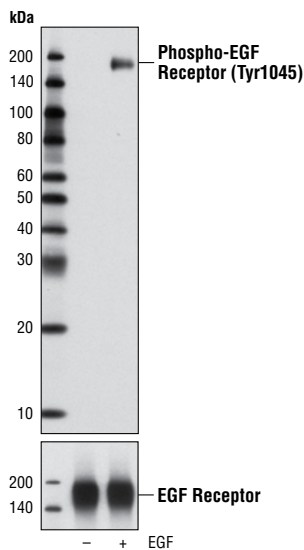
human EGF receptor. EGF Receptor Antibody is produced by immunizing animals with a fusion protein containing the cytoplasmic domain of human EGF receptor. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibodies.

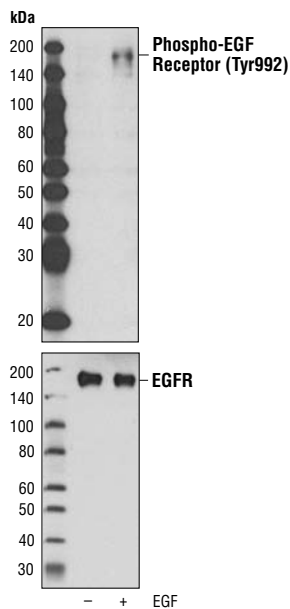
**Recommended Antibody Dilutions:**  
 Western blotting 1:1000

Please visit [www.cellsignaling.com](http://www.cellsignaling.com) for a complete listing of recommended companion products.





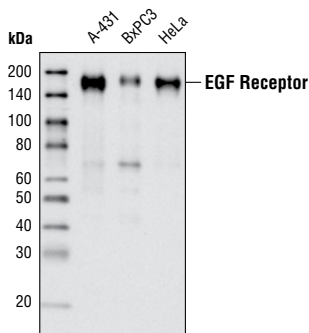
Western blot analysis of extracts from A431 cells, untreated or EGF-stimulated (100 ng/ml), using **Phospho-EGF Receptor (Tyr1045) Antibody #2237** (upper) or EGF Receptor (C74B9) Rabbit mAb #2646 (lower).



Western blot analysis of extracts from A431 cells, untreated or EGF-treated (100 ng/ml), using **Phospho-EGF Receptor (Tyr992) Antibody #2235** (upper) or EGF Receptor Antibody #2232 (lower).

**Background References:**

- (1) Hackel, P.O. et al. (1999) *Curr. Opin. Cell Biol.* 11, 184–189.
- (2) Zwick, E. et al. (1999) *Trends Pharmacol. Sci.* 20, 408–412.
- (3) Cooper, J.A. and Howell, B. (1993) *Cell* 73, 1051–1054.
- (4) Hubbard, S.R. et al. (1994) *Nature* 372, 746–754.
- (5) Biscardi, J.S. et al. (1999) *J. Biol. Chem.* 274, 8335–8343.
- (6) Emlet, D.R. et al. (1997) *J. Biol. Chem.* 272, 4079–4086.
- (7) Levkowitz, G. et al. (1999) *Mol. Cell* 4, 1029–1040.
- (8) Eltenberg, S.A. et al. (1999) *Oncogene* 18, 1855–1866.
- (9) Rojas, M. et al. (1996) *J. Biol. Chem.* 271, 27456–27461.
- (10) Feinmesser, R.L. et al. (1999) *J. Biol. Chem.* 274, 16168–16173.



Western blot analysis of extracts from A-431, BxPC3 and HeLa cells using **EGF Receptor (D38B1) XP™ Rabbit mAb #4267**.

## 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.

- 1X Phosphate Buffered Saline (PBS)
- 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
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 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).
- Nonfat Dry Milk (weight to volume [w/v])
- 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%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- 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%).
- Phototope<sup>®</sup>-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<sup>®</sup> chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- 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.

- 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 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.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- 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.

- Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- 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.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

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

**NOTE:** LumiGLO<sup>®</sup> substrate can be further diluted if signal response is too fast.

- 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<sup>®</sup> incubation and declines over the following 2 hours.