

#2234 Store at -20°C

Phospho-EGF Receptor (Tyr1068) Antibody



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rev. 05/10/16

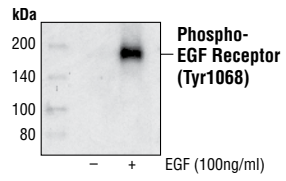
For Research Use Only. Not For Use In Diagnostic Procedures.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IHC-P Endogenous	H, M, R	175 kDa	Rabbit**

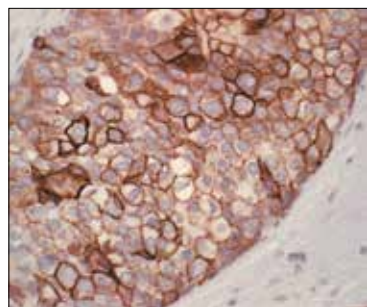
Background: The epidermal growth factor (EGF) receptor is a 170 kDa transmembrane tyrosine kinase that belongs to the HER/ErbB protein family. Ligand binding results in receptor dimerization, autophosphorylation, activation of downstream signaling, internalization and lysosomal degradation (1,2). Phosphorylation 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 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 EGFR 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 of either of these serines results in upregulated EGFR tyrosine autophosphorylation (10).

Specificity/Sensitivity: Phospho-EGF Receptor (Tyr1068) Antibody detects endogenous levels of EGF receptor only when phosphorylated at tyrosine 1068. The antibody may cross-react with other activated EGF receptor family members (e.g. ErbB2), and cross-reacts slightly with activated PDGF receptor.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Tyr1068 of human EGF receptor. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of MDA-468 cells untreated and treated with EGF, using Phospho-EGF Receptor (Tyr1068) Antibody.



Immunohistochemical analysis of paraffin-embedded human breast tumor, showing membrane and cytoplasmic localization, using Phospho-EGF Receptor (Tyr1068) Antibody.

Entrez-Gene ID #1956
Swiss-Prot Acc. #P00533

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 antibody.

*Species cross-reactivity is determined by western blot.
**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:
Western blotting 1:1000
Immunohistochemistry (Paraffin) 1:350†
Unmasking buffer: SignalStain® EDTA Unmasking Solution (10X) #14747
Antibody diluent: SignalStain® Antibody Diluent #8112
Detection reagent: SignalStain® Boost (HRP, Rabbit) #8114
†Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.

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

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.

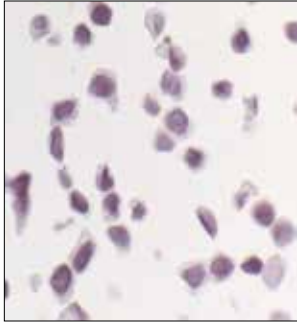
IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

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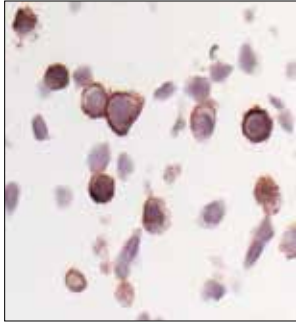
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Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—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.

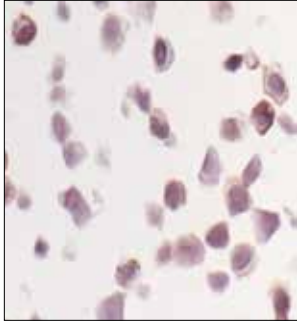
Control



EGF



EGF/Tarceva™



Immunohistochemical analysis of paraffin-embedded MDA-MB-468 cells, untreated (left), EGF treated (middle), or EGF and Tarceva® treated (right), using Phospho-EGF Receptor (Tyr1068) Antibody.