Store at -20°C

#3777

Phospho-EGF Receptor (Tyr1068) (D7A5) XP® Rabbit mAb

☐ Small 100 µl
(10 western blots)

☐ Petite 40 µl
(4 western blots)

Cell Signaling TECHNOLOGY®

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

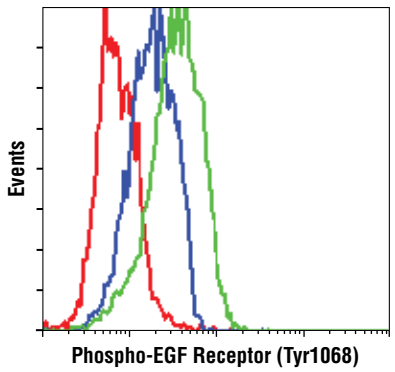
rev. 07/20/11

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

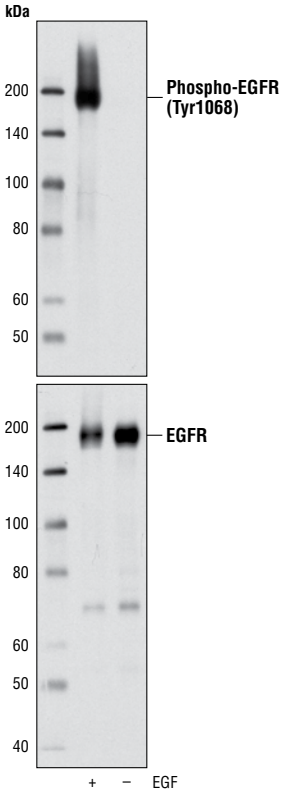
Swiss-Prot Acc. #P54829

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IHC-P, IF-IC, F	H, Mk, M, R	175 kDa	Rabbit IgG**
Endogenous			

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).



Flow cytometric analysis of A549 cells, untreated (blue) or EGF-treated (green), using Phospho-EGF Receptor (Tyr1068) (D7A5) XP® Rabbit mAb compared to concentration matched Rabbit (DA1E) mAb IgG Isotype Control #3900 (red).



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

Specificity/Sensitivity: Phospho-EGF Receptor (Tyr1068) (D7A5) XP® Rabbit mAb detects endogenous EGF receptor only when phosphorylated at Tyr1068. This antibody may cross-react weakly with other tyrosine-phosphorylated proteins.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Tyr1068 of human EGF receptor.

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.*

***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)	1:400†
Unmasking buffer:	EDTA
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114

†*Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.*

Immunofluorescence (IF-IC)	1:800
Flow Cytometry	1:800

For application specific protocols please see the web page for this product at www.cellsignal.com.

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

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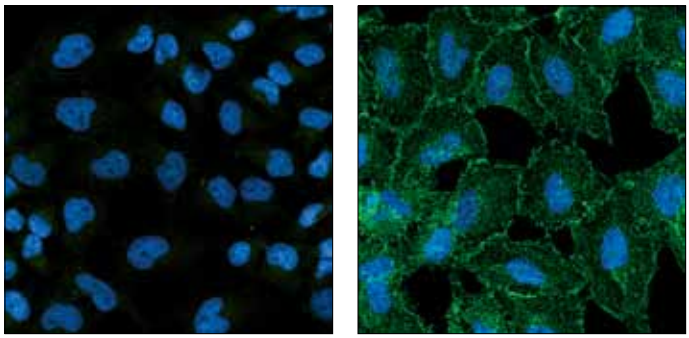
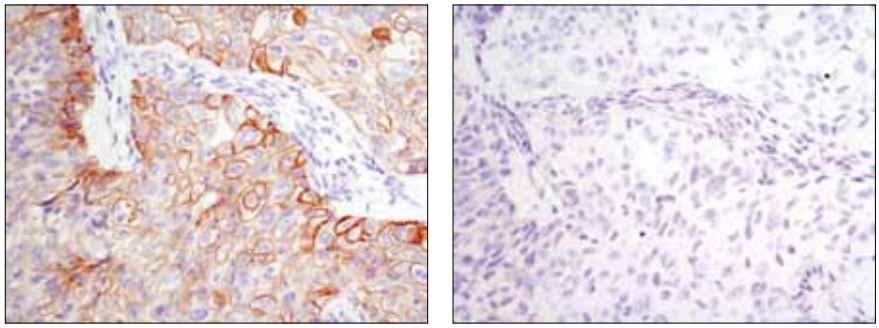
IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

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

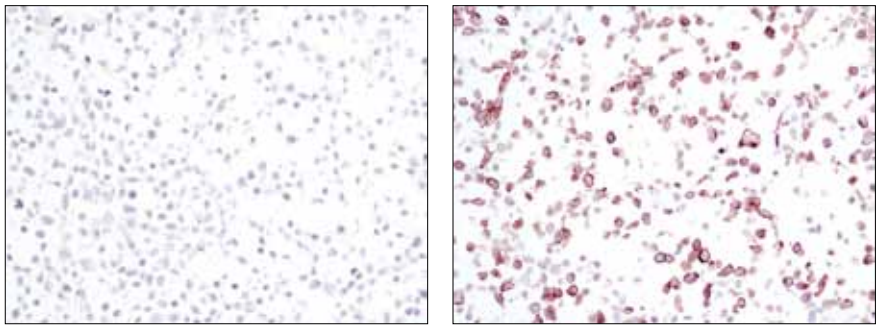
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



Confocal immunofluorescent analysis of HeLa cells, untreated (left) of EGF-treated (right), using Phospho-EGF Receptor (Tyr1068) (D7A5) XP® Rabbit mAb (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



Immunohistochemical analysis of paraffin-embedded HCC827 xenograft, control (left) or λ phosphatase-treated (right), using Phospho-EGF Receptor (Tyr1068) (D7A5) XP® Rabbit mAb.



Immunohistochemical analysis using Phospho-EGF Receptor (Tyr1068) (D7A5) XP® Rabbit mAb on SignalSlide™ Phospho-EGF Receptor IHC Controls #8102 (paraffin-embedded KYSE450 cell pellets, untreated (left) or EGF-treated (right)).

Store at -20°C

#4267

EGF Receptor (D38B1) XP® Rabbit mAb

☑ 100 µl
(10 western blots)

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rev 06/10/11

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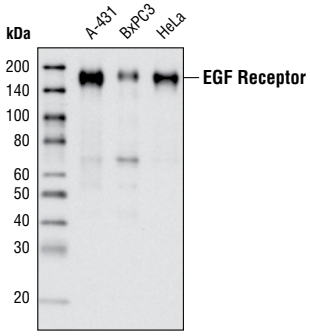
Entrez-Gene ID #1956
Swiss-Prot Acc. #P00533

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P, IF-IC, F Endogenous	H, M, Mk	175 kDa	Rabbit IgG**

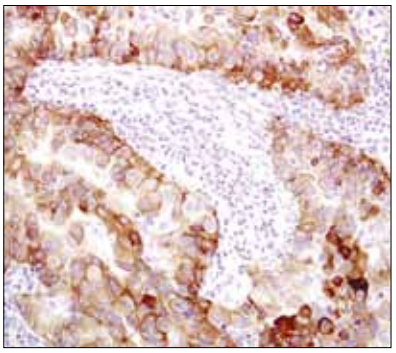
Background: The epidermal growth factor (EGF) receptor is a 170 kDa transmembrane tyrosine kinase that belongs to the HER/ErbB protein family. Ligand binding results in receptor dimerization, autophosphorylation, activation of downstream signaling and lysosomal degradation (1,2). Phosphorylation of EGF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

Specificity/Sensitivity: EGF Receptor (D38B1) XP® Rabbit mAb detects endogenous levels of total EGF receptor protein. The antibody does not cross-react with other proteins of the ErbB family.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a fusion protein containing the cytoplasmic domain of human EGF receptor.



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



Immunohistochemical analysis of paraffin-embedded human lung carcinoma using EGF Receptor (D38B1) XP® Rabbit mAb.

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.*

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

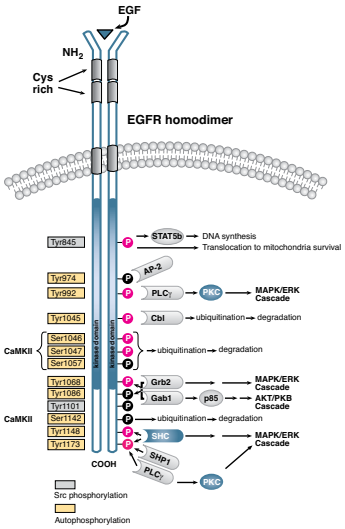
Western blotting	1:1000
Immunoprecipitation	1:100
Immunohistochemistry (Paraffin)	1:50†
Unmasking buffer:	EDTA
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114

†*Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.*

Immunofluorescence (IF-IC)	1:50
Flow Cytometry	1:100

For application specific protocols please see the web page for this product at www.cellsignal.com.

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



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

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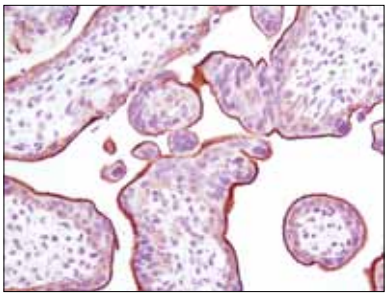
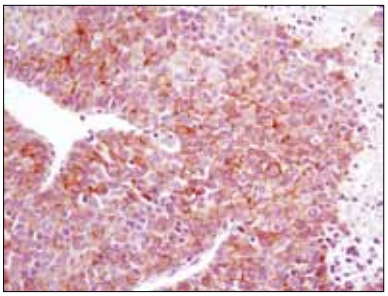


Immunohistochemical analysis of paraffin-embedded human placenta using EGF Receptor (D38B1) XP® Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human hepatocellular carcinoma using EGF Receptor (D38B1) XP® Rabbit mAb.

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.

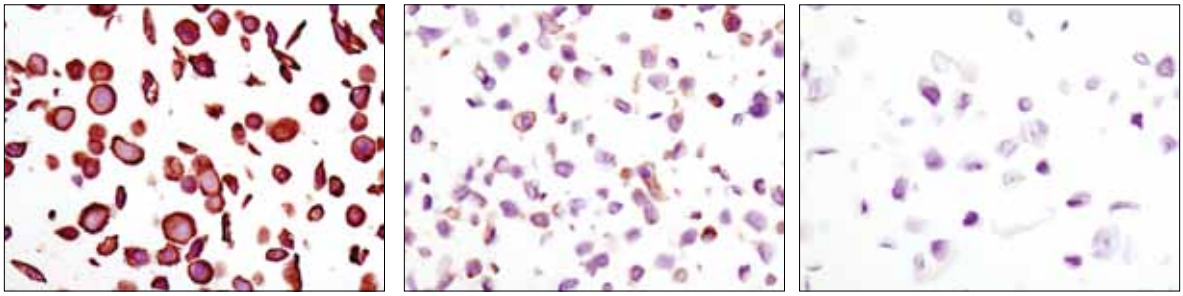
(6) Emlet, D.R. et al. (1997) *J. Biol. Chem.* 272, 4079–4086.

(7) Levkowitz, G. et al. (1999) *Mol. Cell* 4, 1029–1040.

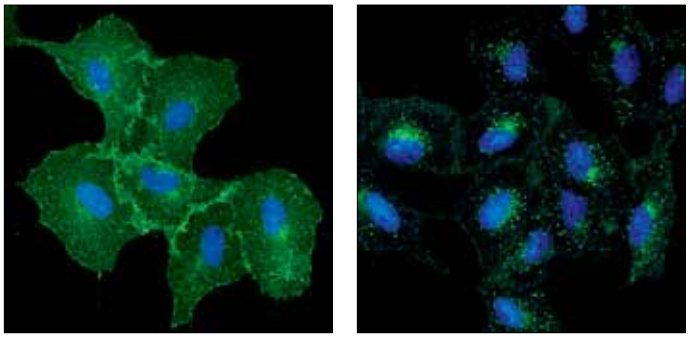
(8) Ettenberg, S.A. et al. (1999) *Oncogene* 18, 1855–1866.

(9) Rojas, M. et al. (1996) *J. Biol. Chem.* 271, 27456–27461.

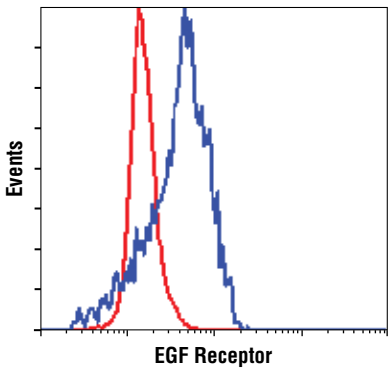
(10) Feinmesser, R.L. et al. (1999) *J. Biol. Chem.* 274, 16168–16173.



Immunohistochemical analysis of paraffin-embedded MDA-MB-468 (amplified EGFR, left), H-T29 (low EGFR, middle) and CAMA-1 (EGFR negative, right) cells using EGF Receptor (D38B1) XP® Rabbit mAb.



Confocal immunofluorescent analysis of A-549 cells, untreated (left) or treated with Epidermal Growth Factor (Human EGF) #9908 (right), using EGF Receptor (D38B1) XP® Rabbit mAb (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



Flow cytometric analysis of Jurkat cells (red) and Kyse70 cells (blue), using EGF Receptor (D38B1) XP® Rabbit mAb.