

Phospho-EGFR (Thr669) Antibody

✓ 100 µl
(10 western blots)

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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.

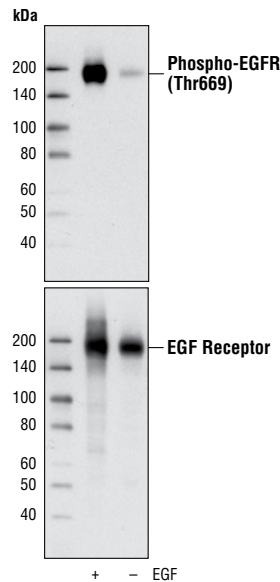
Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W Endogenous	H, R, (M)	175 kDa	Rabbit**

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 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).

Thr669 is a major phosphorylation site on EGF receptor after EGF stimulation. It is phosphorylated by p38 MAP kinase (11). Phosphorylation of the EGF receptor at Thr669 may be involved in regulation of ligand induced receptor internalization by interacting with downstream specific EGF receptor tyrosine kinase substrate(s) (11).

Specificity/Sensitivity: Phospho-EGF Receptor (Thr669) Antibody detects endogenous levels of EGF receptor only when phosphorylated at Thr669. The antibody does not cross-react with other activated EGF receptor family members (e.g. ErbB2).

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr669 of human EGF receptor. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts of A431 cells, untreated or stimulated with EGF, using Phospho-EGF Receptor (Thr669) Antibody (upper) or EGF Receptor Antibody #2232 (lower).

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

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, 184–189.
- (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.
- (11) Winograd-Katz, S.E. and Levitzki, A. (2006) *Oncogene* 25, 7381–7390.

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.