Revision 1

Phospho-EGF Receptor (Thr669) Antibody

Store at -20C

#3056

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W	H R	Endogenous	175	Rabbit	#P00533	1956

Product Usage Information

Application	Dilution
Western Blotting	1:1000

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.

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).

Species predicted to react based on 100% sequence homology

Mouse

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.

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). 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).

Background References

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

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

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

Applications Key

W: Western Blotting

Cross-Reactivity Key

H: Human **R:** Rat

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Limited Uses