

Product Information

Anti-phospho-Epidermal Growth Factor Receptor (pTyr⁹⁹²)

produced in rabbit, affinity isolated antibody

Catalog Number **E1780**

Synonym: Anti-phospho-EGFR (pTyr⁹⁹²)

Product Description

Anti-phospho-Epidermal Growth Factor Receptor (pTyr⁹⁹²) is produced in rabbit using as immunogen a synthetic phosphopeptide derived from the region of EGFR that contains tyrosine 992. The sequence is conserved in human, mouse, and rat (100% homology). Chicken EGFR is 80% homologous. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward either a non-phosphorylated EGFR peptide or a phosphorylated tyrosine peptide, irrespective of the sequence.

Anti-phospho-EGFR (pTyr⁹⁹²) specifically recognizes human epidermal growth factor receptor phosphorylated at Tyr⁹⁹². Mouse, rat, and chicken have not been tested, but are expected to react. The antibody has been used in immunoblotting (~185 kDa).

The epidermal growth factor (EGF) family of receptor tyrosine kinases consists of four receptors, EGFR (ErbB1), ErbB2 (neu), ErbB3 and ErbB4. Members of the EGFR family contain 3 domains: an extracellular domain that is involved in ligand binding and receptor dimerization, a single transmembrane domain and a cytoplasmic domain. EGF exerts its actions by binding to the EGFR. Activation of EGFR results in initiation of diverse cellular pathways. In response to toxic environmental stimuli, or to EGF binding to the receptor, the EGFR forms homo- or heterodimers with other family members.² Each dimeric receptor complex initiates a distinct signaling pathway by recruiting different Src homology 2 (SH2) containing effector proteins. Dimerization results in autophosphorylation on various residues within the cytoplasmic domain, as well as phosphorylation of intracellular substrates, initiating a downstream cascade of events. The activated EGFR dimer forms a complex with the adaptor protein Grb that is coupled to the guanine nucleotide releasing factor, SOS. The Grb-SOS complex can either bind directly to phospho-tyrosine sites or indirectly through Shc.

These protein interactions bring SOS in close proximity to Ras, allowing for Ras activation. This activates the Erk and JNK signaling pathways, which activates transcription factors, such as c-fos, AP-1 and ELK-1, resulting in increased gene expression and cell proliferation.³⁻⁵

Tyr⁹⁹² is situated within the cytoplasmic domain of the receptor. It is an autophosphorylation site, which is phosphorylated to a significantly higher extent by c-Src than by EGFR. Phosphorylation of this site increases the signaling capacity of EGFR.⁵

Reagent

Solution in Dulbecco's phosphate buffered saline (without Mg²⁺ and Ca²⁺) containing 50% glycerol with 1 mg/mL BSA (protease and IgG-free) and 0.05% sodium azide.

The supplied reagent is sufficient for 10 blots.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at -20 °C. Upon initial thawing, freeze the solution in working aliquots for extended storage. Avoid repeated freezing and thawing to prevent denaturing the antibody. Do not store in frost-free freezers. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

Immunoblotting: a recommended working dilution of 1:1,000 is recommended using human epidermoid carcinoma (A431) cells +/- EGF.

Note: In order to obtain the best results in various techniques and preparations, we recommend determining optimal working dilutions by titration.

References

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2. Wells, A., EGF receptor. *Int. J. Biochem. Cell Biol.*, **31**, 637-643 (1999).
3. Quan, X., et al., N terminus of Sos 1 Ras exchange factor: critical roles for the Dbl and plecstrin homology domains. *Mol. Cell Biol.*, **18**, 771-778 (1998).
4. Lanzetti, L., et al., The Eps8 protein coordinates EGF receptor signaling through Rac and trafficking through Rab5. *Nature*, **408**, 374-377 (2000).
5. Lombardo, C.R., et al., *In vitro* phosphorylation of the epidermal growth factor receptor autophosphorylation domain by c-Src: identification of phosphorylation sites and c-Src SH2 domain binding sites. *Biochemistry*, **34**, 16456-16466 (1995).

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