

Product Information

Anti-Phospho-Epidermal Growth Factor Receptor (EGFR) (pTyr¹¹⁴⁸)

Developed in Rabbit, Affinity Isolated Antibody
Product Number **E 1655**

Product Description

Anti-Phospho-Epidermal Growth Factor Receptor (EGFR) (pTyr¹¹⁴⁸) is developed in rabbit using a synthetic phosphorylated peptide derived from the region of EGFR that contains tyrosine 1148 as immunogen. The sequence is conserved in human, mouse, chicken and rat. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preabsorbed to remove any reactivity toward either a non-phosphorylated EGFR peptide or a phosphorylated tyrosine peptide, irrespective of the sequence. Anti- Phospho-EGFR [pTyr¹¹⁴⁸] specifically recognizes epidermal growth factor receptor phosphorylated at tyrosine 1148 (approximately 170 kDa). The antibody detects human EGFR [pTyr¹¹⁴⁸]. Other species have not been tested. It has been used in immunoblotting applications.¹

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 cytoplasmic domain. EGF exerts its actions by binding to the EGFR, a 170 kDa glycoprotein.

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 (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

Tyrosine 1148 is situated within the cytoplasmic domain of the receptor. It is an autophosphorylation site, whose role in EGFR signal transduction is currently under investigation.

Reagent

Anti-Phospho-EGFR [pTyr¹¹⁴⁸] is provided in phosphate buffer, pH 7.4 containing 1 mg/ml BSA (protease and IgG-free) and 0.05% sodium azide. The supplied reagent is sufficient for 10 blots.

Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

Store at -70 °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. The antibody is stable for at least 6 months when stored appropriately.

Product Profile

A recommended working concentration of 0.1 to 1.0 µg/ml is determined by immunoblotting using human epithelial A431 cells stimulated with EGF or mouse hybridoma NIH3T3 cells expressing human EGFR and stimulated with EGF.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

References

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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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