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ProductInformation

Anti-phospho-Epidermal Growth Factor Receptor (EGFR) [pTyr^{1173}]
produced in rabbit, affinity isolated antibody

Catalog Number **E6279**

Product Description

Anti-phospho-Epidermal Growth Factor Receptor (EGFR) [pTyr^{1173}] is produced in rabbit using a synthetic phosphorylated peptide derived from the region of human EGFR that contains tyrosine 1173 as immunogen. The sequence is conserved in mouse and rat. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward non-phosphorylated EGFR.

Anti-phospho-EGFR [pTyr^{1173}] specifically recognizes human EGFR phosphorylated at tyrosine 1173 by immunoblotting.[1] Mouse and rat have not been tested but are expected to react. The antibody has also been used in immunostaining.

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, a 170 kDa protein.

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 auto-phosphorylation 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 phosphotyrosine sites or indirectly through Shc. These protein interactions bring SOS in close proximity to Ras, allowing for Ras activation. This subsequently activates the Erk and JNK signaling pathways that, in turn, activate transcription factors, such as c-fos, AP-1 and ELK-1, increasing gene expression and cell proliferation.[3-5]

Tyr^{1173} is an autophosphorylation site at the extreme C-terminus of the cytoplasmic tail of the receptor. Phosphorylation of this site creates a major binding site for the protein tyrosine phosphatase, SHP1. Binding of SHP-1 to the EGFR correlates with its capacity to dephosphorylate the receptor. The EGFR$_{1171\text{-}1176EpoR}$ mutant, which exhibits elevated SHP-1 binding, is more readily dephosphorylated. [6]

Reagent

Supplied in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3, with 50% glycerol, 1.0 mg/ml BSA (IgG, protease free) and 0.05% sodium azide.

Storage/Stability

Store at –20 °C. For extended storage, upon initial thawing, freeze in working aliquots. Do not store in frost-free freezers. Avoid repeated freezing and thawing to prevent denaturing the antibody. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 6 months when stored appropriately.

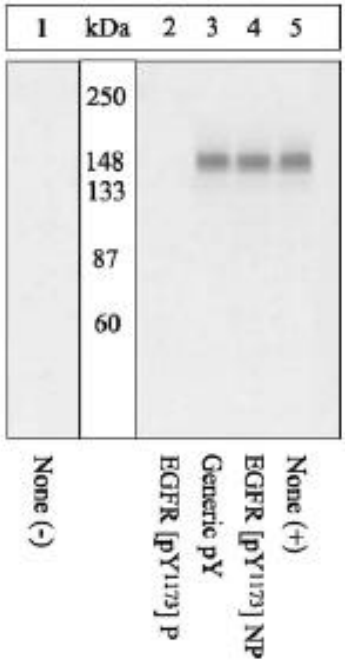
Product Profile

Immunoblotting: a recommended working dilution of 1:1000 is determined using EGF-stimulated and non-stimulated A431 cells.

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

Peptide Competition

1. Extracts prepared from A431 cells were left unstimulated (1) or stimulated with EGF (2-5),
2. Extracts were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
3. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C .
4. The membranes were preincubated as follows:
   - Lane 1 & 5 — no peptide
   - Lane 2 — the phosphopeptide immunogen
   - Lane 3 — a generic phosphotyrosine-containing peptide
   - Lane 4 — the non-phosphopeptide corresponding to the immunogen
5. Following preincubation, all membranes were incubated with EGFR [pTyr^{1148}] antibody for two hours at room temperature in a 1% BSA-TBST buffer,
6. After washing, membranes were incubated with goat F(ab')$_2$ anti-rabbit IgG-HRP and signals were detected using the Pierce SuperSignal® method.



The data show that only the phosphopeptide corresponding to EGFR [pTyr^{1148}] antibody blocks the antibody signal, demonstrating the specificity of the antibody. The data also show the induction of phosphorylation by the addition of EGF.

References

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AH,PHC 07/06-1