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ProductInformation

Anti-phospho-Epidermal Growth Factor Receptor (EGFR) (pTyr^{1086})
Developed in Rabbit, Affinity Isolated Antibody

Product Number **E 1530**

Product Description

Anti-phospho-Epidermal Growth Factor Receptor (EGFR) (pTyr^{1086}) is developed in rabbit using a synthetic phosphorylated peptide derived from the region of human EGFR that contains tyrosine 1086 as immunogen. The sequence is conserved in human, mouse and rat. 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 [pTyr^{1086}] specifically recognizes human epidermal growth factor receptor phosphorylated at tyrosine 1086 (approx. 185 kDa). Mouse and rat have not been tested, but are expected to react. It has been used in immunoblotting applications.[1, 2]

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 1086 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. Extensive c-Src phosphorylation of EGFR promotes its conversion to a form that exhibits high-affinity (K_D = 380 nM) and binding to the SH2 domain of c-Src. The identification of c-Src phosphorylation sequences on EGFR as c-Src SH2 binding sites supports the notion that this interaction plays a significant role in the regulation of growth factor receptor function and signal transduction.[6]

Reagent

The antibody is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3, 50% glycerol with 1.0 mg/mL BSA (IgG, protease free) as a carrier, and 0.05% sodium azide.

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 –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. The antibody is stable for at least 6 months when stored appropriately.

Product Profile
The supplied antibody is sufficient for 10 immunoblots.

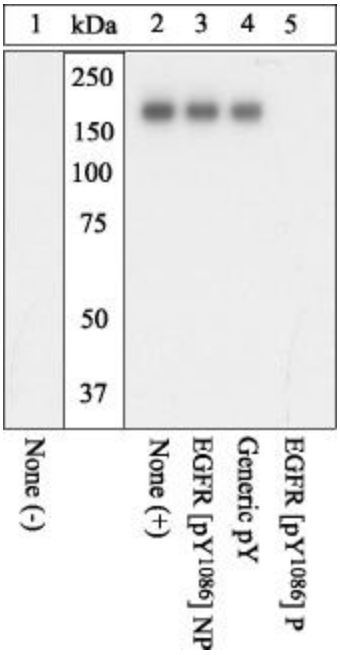
A recommended working dilution of 1:1000 is determined by immunoblotting..

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 of A431 cells unstimulated (Lane 1) or stimulated with 200 ng/mL EGF for 15 minutes (Lanes 2-5) were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
2. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C.
3. After blocking, membranes were preincubated with different peptides as follow:
   - Lane 1,2 no peptide
   - Lane 3 non phosphorylated peptide corresponding to the immunogen
   - Lane 4 a generic phosphotyrosine containing peptide
   - Lane 5 immunogen
4. After preincubation membranes were incubated with EGFR [pTyr^{1086}] antibody for two hours at room temperature in a 1% BSA-TBST buffer.
5. After washing, membranes were incubated with goat F(ab')$_2$ anti-rabbit IgG alkaline phosphatase and signals were detected using the SuperSignal® method.

The data show that only the peptide corresponding to EGFR [pTyr1086] blocks the antibody signal, thereby demonstrating the specificity of the antibody.



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